DEVELOPMENT OF AN EXPERIMENTAL ALBINO RAT (*Rattus norvegicus*) MODEL FOR STUDIES ON SUB-ACUTE BLOOD LOSS ANAEMIA

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ABSTRACT

The objective of this study was to develop an experimental albino rat (Rattus norvegicus) model for studies on sub-acute blood loss anaemia. A total of 160 albino rats of either sexes, between the ages of 10 and 12 weeks were used for the study. One hundred and thirty of the rats were randomly assigned into thirteen groups (groups 1 – 13) of ten each for the first experiment. Baseline values of the haematological indices were determined before the commencement of the study. Rats in group 1 were not bled and served as control. Blood (1 or 2 ml/100g body weight) was removed for specified periods of time from the remaining 12 groups (groups 2 – 13). At the end of the specified period of bleeding for each group, the haematological indices were re-assessed in order to select which group met the target of halving the red blood cell (RBC) count, haemoglobin concentration (Hb) and packed cell volume (PCV) with minimal stress. The selected group was tested for reproducibility in experiment 2 using the remaining thirty rats randomly assigned into three groups (A, B and C). Results showed that in experiment 1, the PCV, Hb and RBC counts of the rat groups that were bled (groups 2 – 13) decreased significantly (p<0.05) after bleeding to varying degrees across the groups based on the volume of blood removed and the duration. Among all the rat groups bled, males of group 12 which were bled 2ml/100g body weight every other day for 20 days ranked topmost among others with the highest reductions in the RBC counts (46.62%), Hb (55.25%) and PCV (46.20%), and no mortality. The results recorded for the two rat groups bled in experiment 2 did not significantly (p>0.05) differ from each other and from that recorded in experiment 1 for the group 12 male rats. Based on the results of the study, removal of 2ml of blood/100g body weight from 10 – 12 weeks old male albino rats every other day for 20 days was recommended for the induction of experimental sub-acute blood loss anaemia.

Keywords: Anaemia, Sub-acute blood loss, Albino rat, *Rattus norvegicus,* Model

INTRODUCTION

Anaemia is a reduction of the red blood cell (RBC) counts and/or haemoglobin concentration (Hb) per unit volume of blood below the normal for the species (Coles, 1986; Ihedioha, 2004; Brockus, 2011). Anaemia is rated a global public health problem by the World Health Organization (WHO) (de Benoist *et al*., 2008; Stevens *et al*., 2013), and it is associated with adverse effects such as increased risk of maternal and child mortality, poor cognitive and

physical development of children and lowered work productivity in adults amongst others (Scholl and Hediger, 1994; Hass and Brownlie, 2001; Zhang *et al*., 2009; Kozuki *et al*., 2012). The global prevalence of anaemia was estimated to be 24.8% (1.62 billion of the world's population affected), with very high prevalence of 47.4% reported for preschool-age children, followed by pregnant women (41.8%) and the least prevalence of 12.7% reported for men (de Benoist *et al*., 2008). A more recent trend study covering 1995 to 2011 had reported a decrease in anaemia prevalence from 47% to 43% in children, 43% to 33% in pregnant women and from 33% to 29% in non-pregnant women (Stevens *et al*., 2013).

Anaemia is caused by blood loss, accelerated erythrocyte destruction, and/or defective erythropoiesis (Coles, 1986; Ihedioha, 2004; Stockham and Scott, 2008; Brockus, 2011). Blood loss anaemia, also called haemorrhagic anaemia, results from severe haemorrhages which may be external (blood is lost from the body) or internal (blood is lost into the body cavities), and acute, sub-acute or chronic (Coles, 1986; Jones *et al.,* 1997; Ihedioha, 2004). Sub-acute and chronic haemorrhages are the types of blood loss that sets in slowly allowing the body to adapt so that hypovolemia does not occur. This types of haemorrhage occur frequently in conditions of parasitism due to internal parasites such as hookworms (*Ankylostoma* sp.), stomach worms (*Haemonchus* sp.*),* nodular worms (*Oesophagustomum* sp.*)* and coccidia (*Eimeria* sp.*)* and external parasites such as ticks, blood sucking lice and fleas (Wells, 1931; Andrews, 1942; Delaune and Mayhew, 1943; Dobson, 1967; Soulsby, 1982; Spencer and Canfield, 1993; Koirnarski *et al.,* 2004). Blood can also be lost continually over time when there are ulcerations of the gastrointestinal tracts, haemorrhagic gastritis and enteritis, inflammatory bowel diseases, mild forms of coagulation defects, bleeding into the genito-urinary tract, and in bleeding into body cavity from a neoplasm (Coles, 1986; Price, 1992; Radostits *et al.,* 1994; Ristic and Stidworthy, 2002; Aster, 2003; Barnert and Messman, 2009). Jones *et al.* (1997) reported that haemophilia and vitamin C deficiency also result in chronic haemorrhagic anaemia.

A model organism is a non-human species studied to understand specific biologic phenomenon when experimentation on humans or other animals is not feasible or ethical (Fields and Johnston, 2005; Govind, 2011). Animal models are indispensable in biomedical research (Govind, 2011). The laboratory rat (*Rattus norvegicus*) was the first mammalian species domesticated for biomedical research (Lindsey, 1979) and had remained a preferred model of human diseases because in many instances the physiology of the rat is a closer approximation to that of humans; the rat is also easier to manipulate and the size makes it easier to handle and make serial blood draws (Gill *et al*., 1989; Jacob, 1999; Ihedioha *et al*., 2004; Iannaccone and Jacob, 2009; Gitig, 2010).

Several anaemia models had been developed. They include canine and mouse models of acute anaemia (Spotswood *et al*., 2005; Liu *et al*., 2009), mouse models of erythropoietin deficiency anaemia (Zeigler *et al*., 2010; Yamazaki *et al*., 2013), iron deficiency anaemia models (Rennie *et al*., 1982; Bhargave and Gabbe, 1984), haemolytic anaemia models (Itano *et al*., 1975; Berger, 2007), mouse models of sickle cell anaemia (Greaves *et al*., 1990; Ryan *et al*., 1997), monkey models of malaria-induced anaemia (Egan *et al*., 2002) and aplastic anaemia models (Scheinberg and Chen, 2013). There is however paucity of information in available literature on sub-acute blood loss anaemia models, yet several food substances, substrates, drugs, chemical combinations and herbal extracts are commonly being tested for their efficacy in the management of sub-acute blood loss anaemia (haematinic effects). The objective of this study was to develop an experimental albino rat model for studies on sub-acute blood loss anaemia and test the developed model for reproducibility. The study shall specifically remove varied small amounts of blood from male and female rats over specified time durations in order to nearly attain a halving of the RBC count, Hb and packed cell volume (PCV), with minimal mortality.

MATERIALS AND METHODS

One hundred and sixty Sprague-Dawley albino rats (*Rattus norvegicus*) of either sexes, between the ages of 10 and 12 weeks were used for the study. This age group was chosen because of their known rate of natural recovery from blood loss anaemia (Refino and Dallman, 1983). The rats were obtained from Laboratory Animal Unit of the Department of Zoology and Environmental Biology, University of Nigeria, Nsukka. They were housed in clean rat cages at the Experimental Animal House of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. The rats were humanely cared for in compliance with the principles of laboratory animal care (NAS, 2011). They were fed *ad libitum* with commercially prepared growers mash (Vital Feeds, Grand Cereals Limited, Jos, Nigeria), and clean drinking water was freely provided for them throughout the period of study.

The study was made up of two experiments – experiment 1 for development of the model and experiment 2 for testing the developed model for reproducibility. The rats were acclimatized for two weeks before each of the experiments commenced. One hundred and thirty rats were used for experiment 1. They were randomly assigned into 13 groups (groups 1 – 13) of ten rats each, made up of 5 males and 5 females kept in separate cages. After the two weeks of acclimatization, baseline values of the erythrocytic profile were determined for all the 13 groups. Group 1 served as the control and no blood was removed from rats in this group. Blood loss was induced in rats in the remaining 12 groups (groups 2 – 13) as follows: group 2 had 1 ml of blood/100g body weight removed daily for 7 days, group 3 had 1 ml of blood/100g body weight removed daily for 10 days, group 4 had 1 ml of blood/100g body weight removed daily for 14 days, group 5 had 1 ml of blood/100g body weight removed every other day for 14 days (7 times), group 6 had 1 ml of blood/100g body weight removed every other day for 20 days (10 times), group 7 had 1 ml of blood/100g body weight removed every other day for 28 days (14 times), group 8 had 2 ml of blood/100g body weight removed daily for 7 days, group 9 had 2 ml of blood/100g body weight removed daily for 10 days, group 10 had 2 ml of blood/100g body weight removed daily for 14 days, group 11 had 2 ml of blood/100g body weight removed every other day for 14 days (7 times), group 12 had 2ml of blood/100g body weight removed every other day for 20 days (10 times) and group 13 had 2ml of blood/100g body weight removed every other day for 28 days (14 times). At the end of the specified period of bleeding for each group, the erythrocytic profile was re-assessed. The erythrocytic profile of the control group (group 1) was also re-assessed at the end of the experiment (day 28).

Thirty male rats were used for experiment 2. They were randomly assigned into three groups (A, B and C) of ten each. Baselines of their erythrocytic profile were determined after two weeks of acclimatization. Based on the outcome of experiment 1, 2ml of blood/100g body weight was removed every other day from rats in groups A and B for 20 days (10 times). Blood was not removed from rats in group C that served as control. After day 20 of the experiment, the erythrocytic profile of rats in all the three groups was re-assessed.

Removal of blood to induce anaemia and collection of blood for haematology was done by the orbital bleeding technique (Bolliger and Everds, 2010). The blood sample for haematology (0.5ml) was dispensed into sample bottles containing potassium ethylene diamine tetra acetic acid (EDTA). The packed cell volume (PCV) was determined by the microhaematocrit method (Thrall and Weiser, 2002), while the haemoglobin concentration was determined by the cyanomethaemoglobin method (Higgins *et al*., 2008). The red blood cell count was conducted following the haemocytometer method (Thrall and Weiser, 2002).

Data obtained from the study were subjected to appropriate statistical analysis using SPSS statistical software. Data from all parameters assessed for the groups were subjected to one way analyses of variance (ANOVA), and variant means were further separated using the least significant difference (LSD) method.

Data generated before bleeding (baseline values) were compared with those generated after bleeding using the student's t-test. Significant difference was accepted at $p < 0.05$, and the results were presented as mean ± SD of means.

RESULTS

In experiment 1, the RBC counts and Hb of all the rat groups that were bled (groups 2 – 13) were significantly ($p<0.05$) reduced after bleeding, but there was no significant change ($p > 0.05$) in the RBC count and Hb of the group 1 (control) rats that were not bled (Table 1 and 2). The greatest percentage reductions in RBC counts were recorded for group 11 females (51.28%), followed by group 9 females (47.74%), then group 12 males (46.62%) and group 8 males (45.85%) (Table 1). Among the rat groups that were bled, percentage reductions in RBC counts were least in the group 7 rats (males – 12.63% and females 21.15%) (Table 1).

Table 1: The red blood cell (RBC) counts of rat groups from which varied quantities of blood were removed for specified periods, compared with a control group that was not bled

Groups[#]	Sex	RBC counts (10^6/µl)		Percentage change (%)
		Before	After	
Group 1 (control – not bled)	Males	8.73 ± 0.98	9.04 ± 0.74	+3.55
	Females	9.33 ± 1.12	9.49 ± 0.53	+1.71
Group 2 (1ml daily for 7 days)	Males*	7.60 ± 0.63	5.10 ± 0.88	-32.89
	Females*	7.22 ± 1.02	5.53 ± 0.30	-23.41
Group 3 (1ml daily for 10 days)	Males*	7.43 ± 0.52	4.44 ± 0.98	-40.24
	Females*	6.93 ± 0.47	4.35 ± 0.45	-37.23
Group 4 (1ml daily for 14 days)	Males*	7.45 ± 1.17	5.10 ± 0.88	-31.54
	Females*	7.54 ± 1.03	4.89 ± 0.30	-35.15
Group 5 (1ml every other day for 14 days)	Males*	8.55 ± 0.42	6.18 ± 0.67	-27.72
	Females*	9.04 ± 0.52	5.80 ± 0.24	-35.84
Group 6 (1ml every other day for 20 days)	Males*	9.21 ± 0.87	7.17 ± 0.77	-22.15
	Females*	9.03 ± 0.93	6.37 ± 0.65	-29.46
Group 7 (1ml every other day for 28 days)	Males*	8.55 ± 0.35	7.47 ± 0.81	-12.63
	Females*	9.41 ± 0.64	7.42 ± 0.49	-21.15
Group 8 (2ml daily for 7 days)	Males*	7.11 ± 0.99	3.85 ± 0.56	-45.85
	Females*	7.44 ± 0.57	4.71 ± 0.89	-36.69
Group 9 (2ml daily for 10 days)	Males*	8.00 ± 0.59	4.37 ± 0.47	-45.38
	Females*	7.75 ± 1.26	4.05 ± 0.68	-47.74
Group 10 (2ml daily for 14 days)	Males*	7.43 ± 1.06	4.06 ± 0.65	-45.36
	Females*	6.77 ± 1.16	4.23 ± 0.76	-37.52
Group 11 (2ml every other day for 14 days)	Males*	9.07 ± 0.29	5.19 ± 0.40	-42.78
	Females*	8.99 ± 0.66	4.38 ± 0.39	-51.28
Group 12 (2ml every other day for 20 days)	Males*	9.03 ± 0.65	4.82 ± 0.34	-46.62
	Females*	9.71 ± 0.74	5.60 ± 0.93	-42.33
Group 13 (2ml every other day for 28 days)	Males*	9.17 ± 0.62	6.58 ± 0.53	-28.24
	Females*	8.88 ± 1.06	6.37 ± 0.57	-28.27

**Asterisk indicates a significant difference before and after bleeding, $p < 0.05$, [#] Volume of blood removed per 100g body weight of rat in bracket for the rat groups bled.*

For the Hb, the greatest percentage reductions were recorded for males of group 10 (67.20%), followed by males of group 9 (57.82%), them males of group 12 (55.25%) and males of group 4 (54.33%) (Table 2). The least percentage reductions in Hb of the rats that were bled was recorded for females of group 7 (16.89%) followed by males of group 2 (18.89%) (Table 2).

Significant ($p<0.05$) reductions in the PCV were recorded for rats in groups 4 – 13 after bleeding, but there were no significant ($p < 0.05$) differences between the PCV before and after bleeding in rats of groups 2 and 3 (Table 3). There was also no significant ($p < 0.05$) change in the mean PCV of the group 1 (control) rats that were not bled (Table 3).

Table 2: The haemoglobin concentration (Hb) of rat groups from which varied quantities of blood were removed for specified periods, compared with a control group that was not bled

Groups[#]	Sex	Hb (g/dl)		Percentage change (%)
		Before	After	
Group 1 (control – not bled)	Males	14.64 ± 1.02	15.42 ± 1.11	+5.33
	Females	14.70 ± 0.82	14.29 ± 0.80	-2.79
Group 2 (1ml daily for 7 days)	Males*	14.82 ± 1.43	12.02 ± 1.19	-18.89
	Females*	16.11 ± 1.07	12.55 ± 0.80	-22.10
Group 3 (1ml daily for 10 days)	Males*	15.25 ± 1.67	8.15 ± 0.70	-46.56
	Females*	16.02 ± 1.08	9.15 ± 1.23	-42.88
Group 4 (1ml daily for 14 days)	Males*	16.38 ± 1.17	7.48 ± 1.68	-54.33
	Females*	15.48 ± 0.93	9.10 ± 0.83	-41.21
Group 5 (1ml every other day for 14 days)	Males*	15.03 ± 1.04	9.66 ± 0.98	-35.73
	Females*	14.65 ± 0.47	9.66 ± 0.69	-34.06
Group 6 (1ml every other day for 20 days)	Males*	15.70 ± 1.01	10.24 ± 1.36	-34.78
	Females*	14.76 ± 0.47	10.23 ± 1.25	-30.69
Group 7 (1ml every other day for 28 days)	Males*	14.64 ± 1.40	11.04 ± 1.25	-24.59
	Females*	15.39 ± 0.78	12.79 ± 0.58	-16.89
Group 8 (2ml daily for 7 days)	Males*	16.06 ± 1.31	8.17 ± 0.75	-49.13
	Females*	15.62 ± 1.66	9.83 ± 1.09	-37.07
Group 9 (2ml daily for 10 days)	Males*	15.93 ± 1.13	6.72 ± 1.32	-57.82
	Females*	14.91 ± 1.89	7.95 ± 0.95	-46.68
Group 10 (2ml daily for 14 days)	Males*	15.70 ± 1.91	5.15 ± 1.05	-67.20
	Females*	14.67 ± 2.13	6.58 ± 0.49	-55.15
Group 11 (2ml every other day for 14 days)	Males*	14.72 ± 1.28	6.87 ± 0.62	-53.33
	Females*	13.79 ± 2.01	7.68 ± 0.76	-44.31
Group 12 (2ml every other day for 20 days)	Males*	15.15 ± 1.54	6.78 ± 1.05	-55.25
	Females*	15.15 ± 0.49	8.77 ± 0.65	-42.11
Group 13 (2ml every other day for 28 days)	Males*	15.81 ± 0.45	8.86 ± 0.81	-43.96
	Females*	14.72 ± 0.85	9.43 ± 1.11	-35.94

**Asterisk indicates a significant difference before and after bleeding, $p < 0.05$; [#] Volume of blood removed per 100g body weight of rat in bracket for the rat groups bled.*

The greatest reductions in percentage PCV were recorded for the group 12 males (46.20%), followed by group 10 males (42.70%), and then group 12 females (39.24%) and group 10 females (33.71%) (Table 3). The least percentage reductions in mean PCV of the rats that were bled was recorded in group 2 males (0.97%) and females (2.88%) (Table 3).

Bleeding-associated mortality was recorded during the experimental period in three females out of the five in group 8, two out of the five of both males and females in group 9, and one out of five of males in group 8, 10 and 13. No mortality was recorded for rats in groups 1, 2, 3, 4, 5, 6, 7, 11 and 12. Bleeding rats every other day was found to be more humane for the rats and convenient for the haematologist than daily removal of blood.

Based on the results recorded above, group 12 males were selected as the group that ranked overall topmost in attaining the target of halving the RBC count, Hb and PCV with minimal mortality – with a reduction of 46.62% of the RBC counts, 55.25% of Hb and 46.20% of the PCV, and no mortality.

In experiment 2 (reproducibility study), the percentage reductions in RBC counts of the group A and B rats (47.71%) and 45.81%, respectively) were comparable to that recorded for the group 12 males (46.62%) in experiment 1 (Table 4). The 55.74% and 55.09% reductions in Hb recorded for the groups A and B rats, respectively were also comparable to the 55.25% reported for the group 12 males in experiment 1 (Table 4). Percentage reductions in PCV of group A and B rats (46.44% and 46.67%, respectively) were also comparable to the 46.20% reported for group 12 males in experiment 1 (Table 4). No significant ($p>0.05$) changes were recorded for group C rats as was the case in the group 1 males in experiment 1 (Table 4).

Table 3: The packed cell volume (PCV) of rat groups from which varied quantities of blood were removed for specified periods, compared with a control group that was not bled

Groups[#]	Sex	PCV (%)		Percentage change (%)
		Before	After	
Group 1 (control – not bled)	Males	47.70 ± 2.28	49.70 ± 1.72	+4.19
	Females	46.80 ± 1.44	47.00 ± 2.12	+0.43
Group 2 (1ml daily for 7 days)	Males	41.10 ± 4.48	40.70 ± 3.27	-0.97
	Females	45.20 ± 2.66	43.90 ± 1.56	-2.88
Group 3 (1ml daily for 10 days)	Males	41.50 ± 2.38	45.00 ± 6.78	+8.44
	Females	46.70 ± 3.21	46.80 ± 7.15	+0.21
Group 4 (1ml daily for 14 days)	Males*	46.30 ± 2.91	33.10 ± 4.45	-28.51
	Females*	47.00 ± 2.00	35.80 ± 3.12	-23.83
Group 5 (1ml every other day for 14 days)	Males*	48.30 ± 2.73	37.60 ± 2.70	-22.15
	Females*	44.70 ± 2.11	35.60 ± 0.82	-20.36
Group 6 (1ml every other day for 20 days)	Males*	51.10 ± 1.95	37.50 ± 3.35	-26.62
	Females*	47.25 ± 2.02	35.80 ±4.40	-24.23
Group 7 (1ml every other day for 28 days)	Males*	47.20 ± 4.38	39.90 ± 3.73	-15.47
	Females*	49.00 ± 2.09	42.00 ± 1.70	-14.29
Group 8 (2ml daily for 7 days)	Males*	45.70 ± 2.99	31.88 ± 2.87	-30.24
	Females*	44.60 ± 4.39	37.00 ± 4.95	-17.04
Group 9 (2ml daily for 10 days)	Males*	45.90 ± 3.75	37.33 ± 5.49	-18.67
	Females*	43.10 ± 3.07	36.50 ± 2.60	-15.31
Group 10 (2ml daily for 14 days)	Males*	44.50 ± 2.29	25.50 ± 4.44	-42.70
	Females*	44.00 ± 2.12	29.17 ± 1.04	-33.71
Group 11 (2ml every other day for 14 days)	Males*	46.20 ± 4.87	31.00 ± 2.74	-32.90
	Females*	45.80 ± 2.17	31.10 ± 3.09	-32.10
Group 12 (2ml every other day for 20 days)	Males*	48.70 ± 2.28	26.20 ± 2.68	-46.20
	Females*	50.20 ± 2.02	30.50 ± 0.98	-39.24
Group 13 (2ml every other day for 28 days)	Males*	48.90 ± 0.89	37.25 ± 3.30	-23.82
	Females*	48.60 ± 1.48	36.20 ± 3.27	-25.51

**Asterisk indicates a significant difference before and after bleeding, $p < 0.05$; [#] Volume of blood removed per 100g body weight of rat in bracket for the rat groups bled*

Table 4: The erythrocytic profile rat groups from which 2ml of blood/100g body weight blood was removed every other day for 20 days (10 times) compared to a control group that was not bled

Groups[#]	Erythrocytic profile of rat		Percentage change (%)
	Before	After	
Red blood cell counts (10^6/µl)			
Group A*	8.97 ± 0.52	4.78 ± 0.33	- 46.71
Group B*	9.06 ± 0.48	4.91 ± 0.36	- 45.81
Group C	9.02 ± 0.46	9.21 ± 0.37	+ 2.11
Haemoglobin concentration (g/dl)			
Group A*	14.87 ± 0.88	6.73 ± 0.72	- 54.74
Group B*	15.23 ± 1.01	6.84 ± 0.93	- 55.09
Group C	15.01 ± 0.96	15.14 ± 1.00	+ 0.20
Packed cell volume (%)			
Group A*	47.80 ± 1.25	25.60 ± 1.64	- 46.44
Group B*	49.50 ± 1.66	27.40 ± 1.72	- 46.67
Group C	48.40 ± 1.41	49.60 ± 1.68	+ 1.65

**Asterisk indicates a significant difference before and after bleeding, $p < 0.05$, [#] Rats in groups A and B were bled 2ml/100g body weight for 20 days (bleeding 10 times) while group C rats were the control group which were not bled.*

No mortality was recorded for any of the rat groups (A, B and C) all through the experiment 2.

DISCUSSION

The significant reductions in the RBC counts, Hb and PCV of the rat groups that were bled is a confirmation that blood loss as applied in this study induced anaemia. The absence of significant changes in the mean PCV of the rat groups bled 1ml of blood/100g body weight for 7 and 10 days (groups 2 and 3 in experiment 1) despite a significant reduction in their RBC counts and Hb is believed to be due to the fact that the PCV value is usually dependent on the number and size of erythrocytes in circulation; thus even with a reduction in number, a massive amount of large sized immature erythrocytes (macrocytes) pushed into the circulation as an response to blood loss can mask off the effects of the reduced RBC numbers and lead to a relative high PCV (Quinto *et al*., 2006; Carneiro *et al*., 2007; Brockus, 2011).

The greater percentage reductions in RBC counts recorded in the rat groups bled 2ml/100g body weight relative to the ones bled 1ml/100g body weight was expected because the magnitude of anaemia had been known to correlate to the volume of blood lost (Salisbury *et al*., 2011). It was worth noting that the group 13 rats (experiment 1) bled 2ml/100g body weight every other day for 28 days had a lower percentage reduction in RBC counts when compared to other rat groups from which 2ml of blood/100g body weight was removed (groups 8 – 12). It is thought that the extended period of bleeding (28 days) gave the opportunity for the bone marrow to respond far more than as recorded for other groups in that category pushing into the circulation higher number of immature erythrocytes (macrocytes) with low Hb content (Christian, 2010), such that even with a great percentage reduction in Hb, their percentage reduction in RBC counts and PCV were comparably low.

The higher frequency of bleeding-associated mortality recorded in the rat groups bled 2ml/100g body weight daily when compared to all other rat groups is believed to be due to the combined effects of higher volume of blood (2ml) removed and the relative rapidity (daily bleeding) of the blood loss; those bled 2ml every other day had some time to recuperate from each bleeding while those bled only 1ml/100g body weight that recorded no mortality may not have suffered reasonable enough blood-loss stress that will lead to death.

The selection of group 12 males that were bled 2ml/100g body weight every other day for 20 days (10 times) was based on this group featuring consistently among the top three groups that had the greatest percentage reductions in the means of their RBC counts, Hb and PCV, and that no bleeding-associated deaths were recorded in this group. The propriety of the choice was confirmed in experiment 2 as the rat groups that were bled in this experiment (Groups A and B) had percentage reductions in their mean RBC counts, Hb and PCV that were comparable to that of group 12 males of experiment 1 and also had no mortality. The results of experiment 2 also confirmed the reproducibility of the selected procedure.

Based on the results of this study, it was concluded that removal of 2ml of blood/100g body weight from 10 – 12 week old albino rats every other day for 20 days (bleeding 10 times) led to attainment of nearly halving the RBC count, Hb and PCV with no mortality. It was recommended that removal of 2ml of blood/100g body weight from 10 – 12 week old male albino rats every other day for 20 days (bleeding 10 times) be adopted as model for inducing experimental sub-acute blood loss anaemia.

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REFERENCES

ANDREWS, J. S. (1942). Stomach worm (*Haemonchus contortus)* infection in lambs and its relation to gastric

haemorrhage and general pathology. *Journal of Agriculture Research*, 65: 1 – 18.

ASTER, J. C. (2003). The haematopoietic and lymphoid system. Pages 395 – 452. *In:* KUMAR, V., COTRAN, R. S. and ROBBINS, S. L. (Eds.), *Robbins Basic Pathology*, Seventh Edition, Saunders, Philadelphia.

BARNERT, J. and MESSMAN, H. (2009). Diagnosis and management of lower gastrointestinal bleeding. *Nature Reviews Gastroenterology and Hepatology*, 6: 637 – 647.

BERGER, J. (2007). Phenylhydrazine haemotoxicity. *Journal of Applied Biomedicine*, 5: 125 – 130.

BHARGAVE, M. and GABBE, E. E. (1984). Chronic experimental iron losses in rat not leading to overt iron deficiency: a model for the regulation of the whole-body iron balance in an iron-replete condition. *Journal of Nutrition*, 114: 1060 – 1069.

BOLLIGER, A. P. and EVERDS, N. E. (2010). Haematology of laboratory rodents: mouse (*Mus musculus*) and rat (*Rattus norvegicus*). Pages 852 – 862. *In:* WEISS, D. J. and WARDROP, K. J. (Eds.), *Schalm's Veterinary Haematology*, Sixth Edition, Wiley-Blackwell, Iowa.

BROCKUS, C. W. (2011). Erythrocytes. Pages 3 – 44. *In:* LATIMER, K. S. (Ed.), *Duncan and Prasse's Veterinary Laboratory Medicine: Clinical Pathology*, Fifth Edition, Wiley-Blackwell, West Sussex, United Kingdom.

CARNEIRO, I. A., DRAKELEY, C. J., OWUSU-AGYEI, S., MMBANDO, B. and CHANDRAMOHAN, D. (2007) Haemoglobin and haematocrit: is the threefold conversion valid for assessing anaemia in malaria-endemic settings? *Malaria Journal,* 6: 67.

COLES, E. H. (1986). *Veterinary Clinical Pathology*, Fourth Edition, W. B. Saunders, Philadelphia.

CHRISTIAN, J. A. (2010). Erythrokinetics and erythrocyte destruction. Pages 136 – 143. *In:* WEISS, D. J. and WARDROP, K. J. (Eds.) *Schalm's Veterinary Haematology*, Sixth Edition, Wiley-Blackwell, Iowa, USA.

DE BENOIST, B., MCLEAN, E., EGLI, I. and COGSWELL, M. (2008). *Worldwide Prevalence of Anaemia 1993 – 2005*. WHO Global Database on Anaemia. World Health Organization (WHO), Geneva, Switzerland.

DELAUNE, E. T. and MAYHEW, R. L. (1943). Studies on bovine gastro-intestinal parasites : VI. The blood picture in stomach worm (*Haemonchus contortus)* infections. *Transactions of the American Microscopical Society*, 162: 179 – 193.

DOBSON, C. (1967). Pathologic changes associated with *Oesophagostomum columbianum* infestations in sheep: Haematological observations on control worm-free and experimental infested sheep. *Australian Journal of Agricultural Research*, 18: 523 – 538.

EGAN, A. F., FABUCCI, M. E., SAUL, A., KASLOW, D. C. and MILLER, L. H. (2002). Aotus New World monkeys: model for studying malaria-induced anaemia. *Blood*, 99(10): 3863 – 3866.

FIELDS, S. and JOHNSTON, M. (2005). Cell biology. Whither model organism research? *Science*, 307(5717): 1885 – 1886.

GILL, I. I. I., SMITH, T. G., WISSLER, R. and KUNZ, H. (1989). The rat as an experimental animal. *Science*, 245: 269 – 276.

GITIG, D. (2010). Rats: and old model made new. *Biotechniques*, 48: 267 – 271.

GOVIND, P. (2011). Model organisms used in molecular biology or medical research. *International Research Journal of Pharmacy*, 2(11): 62 – 65.

GREAVES, D. R., FRASER, P., VIDAL, M. A., HEDGES, M. J., ROPERS, D., LUZZATTO, L and GROSVELD, F. (1990) A transgenic mouse model of sickle cell disorder. *Nature*, 343: 183 – 185.

HAAS, J. D. and BROWNLIE, T. (2001). Iron deficiency and reduced work capacity: a critical review of the research to

determine a causal relationship. *Journal of Nutrition,* 131(2S-2): 676S – 690S.

HIGGINS, T., BEUTLER, E. and DOUMAS, B. T. (2008). Measurement of haemoglobin in blood. Pages 514 – 515. *In:* BURTIS, C. A., ASHWOOD, E. R. and BRUNS, D. E. (Eds.), *Tietz Fundamentals of Clinical Chemistry*, Sixth Edition, Saunders Elsevier, Missouri.

IANNACCONE, P. M. and JACOB, H. J. (2009). Rats! *Disease Models and Mechanisms*, 2: 206 – 210.

IHEDIOHA, J. I. (2004). Haematopoietic system. Pages 107 – 160. *In:* IHEDIOHA, J. I. and CHINEME, C. N. (Eds.), *Fundamentals of Systemic Veterinary Pathology,* Volume 1, Great AP Express Publishers, Nigeria.

IHEDIOHA, J. I., OKAFOR, C. and IHEDIOHA, T. E. (2004). The haematological profile of the Sprague-Dawley out bred albino rat in Nsukka, Nigeria. *Animal Research International*, 1(2): 125 – 132.

ITANO, H. A., HIROTA, K. and HOSOKAWA, K. (1975). Mechanisms of induction of haemolytic anaemia by phenylhydrazine. *Nature*, 256: 665 – 667.

JACOB, H. J. (1999). Functional genomics and rat models. *Genome Research*, 9: 1013 – 1016.

JONES, T. C., HUNT, R. D. and KING, N. W. (1997). *Veterinary Pathology*, 6th Edition. William and Wilkins, Baltimore, Pages 168 – 171.

KOINARSKI, V., ANGELOV, G. and LALEV, M. (2001). Haematologic and blood electrolyte changes in broiler chickens experimentally Invaded with *Eimeria tenella. Bulgarian Journal of Veterinary Medicine*, 4: 237 – 242.

KOZUKI, N., LEE, A. C. and KATZ, J. (2012). Moderate to severe, but not mild, maternal anemia is associated with increased risk of small-for-gestational-age outcomes. *Journal of Nutrition,* 142: 358 – 362.

LINDSEY, J. R. (1979). Historical foundations. Pages 1 – 36. *In:* BAKER, H. J., LINDSEY, J. R. and WEISBROTH, S. H. (Eds.), *The Laboratory Rat*. Academic Press, New York.

LIU, Q., WANG, J., CUI, J., YANG, Z. and DU, G. (2009). A novel acute anaemia model for pharmacological research in mice by compelled acute exercise. *Acta Pharmacologica Sinica*, 30: 1643 - 1647.

NAS (2011). *Guide for the Care and Use of Laboratory Animals*. Eighth Edition, National Academy of Sciences (NAS), National Academy Press, Washington DC.

PRICE, A. B. (1992). Inflammatory bowel disease. Pages 1234 – 1254. *In:* MCGEE, J. O. D., ISAACSON, P. G. and WRIGHT, N. A. (Eds.), *Oxford Textbook of Pathology*, Oxford University Press, Oxford.

QUINTO, L., APONTE, J. J., MENENDEZ, C., SACARLAL, J., AIDE, P., ESPASA, M., MANDOMANDO, I., GUINOVART, C., MACETE, E., HIRT, R., URASSA, H., NAVIA, M. M., THOMPSON, R. and ALONSO, P. L. (2006) Relationship between haemoglobin and haematocrit in the definition of anaemia. *Tropical Medicine and International Health*, 11: 1295 – 1302.

RADOSTITS, O. M., BLOOD, D. C. and GAY, C. C. (1994). Diseases of the blood and blood forming organs. Pages 370 – 390. *In:* RADOSTITS, O. M., BLOOD, D. C. and GAY, C. C. (Eds.) *Veterinary Medicine - A Textbook of Diseases of Cattle, Sheep, Pigs, Goats and Horses*. Eighth Edition, Bailliere Tindall, London.

REFINO, C. J. and DALLMAN, P. R. (1983). Rate of repair of iron deficiency anemia and blood loss anemia in young and mature rats. *American Journal of Clinical Nutrition*, 37: 904 – 909.

RENNIE, J. S., MACDONALD, D. G. and DOUGLAS, T. A. (1982). Experimental iron deficiency in the Syrian hamstar (*Mesocricatus auratus). Laboratory Animals*, 16: 14 – 16.

RISTIC, J. M. and STIDWORTHY, M. F. (2002). Two cases of severe iron deficiency due to inflammatory bowel disease in the

dog. *Journal of Small Animal Practice*, 43: 80 – 83.

RYAN, T. M., CIAVATTA, D. J. and TOWNES, T. M. (1997). Knockout transgenic mouse model of sickle cell disease. *Science*, 278: 873 – 876.

SALISBURY, A. C., REID, K. J., ALEXANDER, K. P., MASOUDI, F. A., LAI, S., CHAN, P. S., BACH, R. G., WANG, T. Y., SPERTUS, J. A. and KOSIBOROD, M. (2011). Diagnostic blood loss from phlebotomy and hospital-acquired anaemia during acute myocardial infarction. *Archives of Internal Medicine*, 171(18): 1646 – 1653.

SCHEINBERG, P. and CHEN, J. (2013). Aplastic anaemia: what we learned from animal models and from the clinic. *Seminars in Hematology*, 50(2):156 – 164.

SCHOLL, T. O. and HEDIGER, M. L. (1994). Anemia and iron-deficiency anemia: compilation of data on pregnancy outcome. *American Journal of Clinical Nutrition*, 59: 492S – 500S.

SOULSBY, E. J. L. (1982). *Helminths, Arthropods and Protozoa of Domestic Animals*. Seventh Edition, Bailliere and Tindall, London.

SPENCER, A. J. and CANFIELD, P. J. (1993). Haematological characterization of heavy tick infestation in koalas (*Phascolarctos cinereus*). *Comparative Haematology International*, 3: 225 – 229.

SPOTSWOODS, T. C., KIRBERGER, R. M., KOMA, L. M. P. K., REYERS, F. and VANDER DER MERWE, L. (2005). A canine normovolaemic acute anaemia model. *Onderstepoort Journal of Veterinary Research*, 72: 135 – 143.

STEVENS, G. A., FINUCANE, M. M., DE-REGIL, L. M., PACIOREK, C. J., FLAXMAN, S. R., BRANCA, F., PENA-ROSAS, J. P., BHUTTA, Z. A. and EZZATI, M. ON BEHALF OF NUTRITION IMPACT MODEL STUDY GROUP (ANAEMIA). (2013). Global, regional, and national trends in haemoglobin concentration and prevalence of total and severe anaemia in children and pregnant and non-pregnant women for 1995 – 2011: a systematic analysis of population-representative data. *Lancet Global Health*, 1: e16 – 25.

STOCKHAM, S. L., and SCOTT, M. A. (2008). *Fundamentals of Veterinary Clinical Pathology*. Second Edition, Blackwell Publishing, Iowa, USA.

THRALL, M. A. and WEISER, M. G. (2002). Haematology. Pages 29 – 74. *In:* HENDRIX, C. M. (Ed.), *Laboratory Procedures for Veterinary Technicians*. Fourth Edition, Mosby Incorporated, Missouri, USA.

WELLS, H. S. (1931). Observations on the blood sucking activities of the hookworm, *Ankylostoma caninum*. *Journal of Parasitology*, 17: 167 – 182.

YAMAZAKI, S., SOUMA, T., HIRANO, I., PAN, X., MINEGISHI, N., SUZUKI, N. A. and YAMAMOTO, M. (2013). A mouse model of adult-onset anaemia due to erythropoietin deficiency. *Nature Communications*, 4: 1950.

ZEIGLER, B. M., VAJDOS, J., QIN, W., LOVERRO, L. and NISS, K. (2010). A mouse model for an erythropoietin-deficiency anaemia. *Disease Models and Mechanisms*, 3: 763 – 772.

ZHANG, Q., ANANTH C. V., RHOADS, G. G. and LI, Z. (2009). The impact of maternal anemia on perinatal mortality: a population-based, prospective cohort study in China. *Annals of Epidemiology,* 19: 793 – 99.