ABSTRACT

**Background and Objectives:** Acute haemorrhage is a major cause of morbidity and mortality in surgery and trauma due to ischaemic lesions followed by multiple organ dysfunction. After haemorrhage, compensatory redistribution of blood volume may occur to more vital and survival organs. The objective of the study was evaluation of thermoregulation and haematological responses to acute haemorrhage in Nubian goats.

**Materials and Methods:** The study used 6 female mature goats, weighing 18.0 kg on average. The goats were subjected to 40% bleeding. The rectal temperature (Tr), respiration rate (RR) and heart rate (HR) were monitored before bleeding and then immediately after bleeding and at 1, 2, 3, 4, 5, 6, 24 hrs post-bleeding. Venous blood samples were collected before and then immediately after bleeding and at 6, 24, 48, 72 hrs post-bleeding. The haemoglobin concentration (Hb), packed cell volume (PCV), total leukocyte count (TLC), differential leukocyte count (DLC), serum total protein, albumin, and concentrations of sodium (Na) were determined.

**Results:** The post-bleeding values of Tr, RR and HR were significantly higher compared to the pre-bleeding values. The post-bleeding values of PCV, Hb concentration, erythrocyte count and TLC...
were lower compared to prebleeding values. The ratios of lymphocytes, monocytes and eosinophils decreased, whereas the neutrophil ratio increased post-bleeding. The values of serum total protein, albumin and serum Na concentrations decreased post-bleeding.

**Conclusion:** The studies indicate that the goat can be adopted as a valid research model in investigations of experimental haemorrhage. The findings have implications in the fields of veterinary haematology and surgery and comparative medicine.

**Keywords:** Goat; acute haemorrhage; thermoregulation; blood constituents.

### 1. INTRODUCTION

Acute haemorrhage that may lead to hypovolaemic shock, is an abnormal physiological state characterized by hypotension caused by the recruitment of extracellular fluids[1,2]. Bleeding of 15 to 20% of total blood volume is clinically detectable, while 30 to 40% bleeding causes life-threatening circulatory failure [3]. Decreases in circulating blood volume during haemorrhage can depress cardiac output and severe haemorrhage impairs the delivery of oxygen and nutrients to meet the metabolic needs of the tissues producing a state of shock [4]. This usually leads to the reduction of available oxygen to tissue cells, hypothermia, metabolic acidosis[5,6] and cardiac and respiratory arrest [7].

The primary physiologic response to compensate for haemorrhagic shock is via the sympathetic nervous system; baroreceptors in the carotid sinus and chemoreceptors sense hypovolaemia and hypoxaemia, respectively [8]. Also, endocrine-mediated mechanisms help to maintain perfusion in shock; the release of catecholamines activates α receptor in the peripheral vascular smooth muscle causing peripheral vasoconstriction [9]. Proportional increase in heart rate (HR) and a decrease in mean arterial pressure (MAP) have been reported in animals exposed to haemorrhage [10,11,12]. The cause of death in animals subjected to haemorrhagic shock was reported to be cardiac arrhythmia [13].

Recently, studies on laboratory animals have been conducted to obtain a better evaluation of haemodynamic and metabolic parameters which occur during acute haemorrhage. The goat may experience considerable blood loss due to trauma and haemorrhage and is relatively resistant to dehydration and diseases. The current study presents a model, based on our previous study on haemorrhage [14]. Goats could represent an appropriate model to investigate surgical interventions in veterinary medicine. This study aimed to assess the acute phase changes in thermoregulation, heart rate and blood constituents in response to 40% acute haemorrhage in Nubian goats.

### 2. MATERIALS AND METHODS

#### 2.1 Animals and Diet

The study used 6 female mature Nubian goats, weighing 18.0 kg on average, that were considered healthy after physical examination and performance of complete haemogram. The animals were non-gestating and non-lactating and were kept in animal pens for an adaptation period of 2 weeks. During the experimental period, the animals were fed alfalfa hay (CP:18%, ME:7.9 MJ/kg) and were offered tap water ad libitum. The study was performed during late winter at the Department of physiology.

#### 2.2 Experimental Design

For all animals, the baseline values of thermoregulation parameters, heart rate(HR) and haematological indices were determined. The goats were subjected to 40% bleeding. Graduated blood collection bags were used to withdraw the specific volume of blood from the jugular vein. Thermoregulation and HR values were obtained for 24 hrs. The haematological responses to haemorrhage were monitored for 72 hrs.

#### 2.3 Thermoregulation and heart rate (HR) measurements

The measurements of rectal temperature (Tr) of the goats were made to the nearest ± 0.1°C using a certified mercury-in-glass clinical thermometer (Hartman-UK). The respiration rate (RR) was measured by visually counting the flank movements. The heart rate (HR) was measured monitoring with the aid of a stethoscope and stopwatch.
2.4 Blood Volume and Blood Sampling

The total blood volume was measured utilizing plasma volume determined by Evans blue dye [15]. Blood samples were taken using disposable syringes before 1 day, immediately before and after bleeding and at 6, 24, 48, 72 hrs post bleeding. Immediately 1 ml of blood volume was transferred to a clean dry test tube containing (Na₂-EDTA) for blood analysis. The rest of the blood was allowed to stay for 2 hr at room temperature and then centrifuged at 3000 r.p.m. for 15 min. Serum samples were pipetted into clean vials and immediately frozen at -20°C for subsequent analysis.

2.5 Blood and Serum Analysis

The haemoglobin concentration (Hb), packed cell volume (PCV), total leukocyte count(TLC), differential leukocyte count(DLC) and blood indices were determined according to the standard methods [16]. The concentrations of serum total protein and albumin were determined by colorimetric methods using kits (Spinreact, S.A. Spain). The concentration of serum sodium (Na) was determined by flame photometer technique [17].

2.6 Statistical Analysis

The experimental data were subjected to appropriate analysis of one way ANOVA test using the SAS package (1998) [18]. The data were presented as mean ± standard deviation (SD) and the P<0.05 was considered statistically significant.

3. RESULTS

3.1 Thermoregulation and HR

The effect of haemorrhage on Tr is shown in Fig.1. The initial values of Tr in the experimental animal was 38.8±0.22°C. For all animals Tr showed significant (P<0.01) increase immediately after haemorrhage. Thereafter, the animals showed a progressive decline in Tr until 2 hr and then showed a slight elevation that was maintained until 24 hr. Fig. 2 shows that the initial mean values of RR for the experimental animal was 28±4.66 breaths/min. The animals showed significantly (P<0.001) higher mean values of RR compared to the initial values immediately post-bleeding and at 1, 2, 3, 4, 5, 6 hrs after haemorrhage. The RR values returned to normal after 24 hrs in goats.

Fig. 3 shows that the initial mean values of HR in animals was 76±6.32 beats/min. There was a significant (P<0.01) increase in HR immediately following bleeding and at 2, 3, 4, 5 hrs after haemorrhage. The mean HR values returned to normal after 24hrs in all experimental goats.

3.2 Erythrocytic Parameters

The responses of PCV are shown in Fig. 4. The initial mean value was 29.4±2.51%. Immediately following haemorrhage, there was no significant change in PCV level. Then, there was progressive and significant (P< 0.001) decline in PCV values at 6, 24, 48 and 72 hrs.

Fig. 5 shows that the initial pre-haemorrhage value of Hb was 11.7±1.28 g/dL. The general pattern of Hb values presented in Fig. 5 indicates significant (P<0.001) decrease immediately post haemorrhage and at 6, 24, 48, and 72hrs post-haemorrhage.

Fig. 6 indicates that the initial value of erythrocyte count was 12.3 X10¹² ±1.58/µL. There was no significant change immediately following haemorrhage. The erythrocytic count decreased significantly (P<0.05) at 6, 48 and 72hrs and significantly(P<0.01) at 24hrs post-haemorrhage.

3.3 Leukocytic Parameters

The effect of haemorrhage on total leukocyte count (TLC) is shown in Fig. 7. The initial pre-bleeding value was 9.6 X10³ ±1.78 /µL. There was a significant decrease (P<0.01) in TLC value immediately post-haemorrhage. Thereafter, there was significant (P<0.05) increase in TLC at 6 and 24hrs, then re-established normal values of TLC after 48 hrs.

Figs. 8, 9, 10 and 11 show the effect of haemorrhage on the ratios of lymphocytes, neutrophils, monocytes and eosinophils, respectively. The lymphocyte ratio was significantly (P<0.01) lower compared to pre-bleeding values at 6, 24, 48 and 72 hrs post-bleeding (Fig. 8), while the neutrophil ratio increased significantly (P<0.01) compared to pre-bleeding values at 6, 24 hrs and increased significantly (P<0.05) at 48 and 72 hrs post haemorrhage (Fig. 9). The monocyte ratio decreased immediately after bleeding, lower values were maintained at 72 hrs post-haemorrhage (Fig. 10). The eosinophil ratio decreased significantly (P<0.05) at 6 and 24hrs post-bleeding, then increased to maintain normal value at 72 hrs (Fig.11).
3.4 Serum Analysis

Fig. 12 shows that the initial total protein mean value was 7.05 ± 0.68 g/dL. The values showed a decrease posthaemorrhage. The decline was significant (P<0.01) at 6, 24, 48 hrs and attained 4.6 g/dL at 72 hrs post-bleeding.

The effect of acute haemorrhage on serum albumin concentration is shown in Fig.13. The initial value was 3.1±0.33g/dL. The albumin concentration decreased significantly (P<0.05) at 6 and 48hrs post-haemorrhage and maintained lower level compared to pre-bleeding values until the end of the experimental period.

Fig.14 shows the effect of haemorrhage on serum Na concentration. The initial mean value was 128±1.92 mEqL⁻¹. The value declined significantly (P<0.05) at 6, 24, 48 and then increased at 72 hrs post-bleeding.

**Fig. 1. Effect of acute haemorrhage on rectal temperature (Tr) in Nubian goats**

**Fig. 2. Effect of acute haemorrhage on respiratory rate (RR) in Nubian goats**
Fig. 3. Effect of acute haemorrhage on heart rate (HR) in Nubian goats

Fig. 4. Effect of acute haemorrhage on packed cell volume (PCV) in Nubian goats

Fig. 5. Effect of acute haemorrhage on haemoglobin concentration (Hb) in Nubian goats
Fig. 6. Effect of acute haemorrhage on erythrocyte count in Nubian goats

Fig. 7. Effect of acute haemorrhage on total leukocyte count (TLC) in Nubian goats

Fig. 8. Effect of acute haemorrhage on lymphocyte ratio in Nubian goats
Fig. 9. Effect of acute haemorrhage on neutrophil ratio in Nubian goats

Fig. 10. Effect of acute haemorrhage on monocyte ratio in Nubian goats

Fig. 11. Effect of acute haemorrhage on eosinophil ratio in Nubian goats
Fig. 12. Effect of acute haemorrhage on serum total protein (TP) concentration in Nubian goats

Fig. 13. Effect of acute haemorrhage on serum albumin concentration in Nubian goats

Fig. 14. Effect of acute haemorrhage on serum sodium (Na) concentration in Nubian goats
4. DISCUSSION

In this study, the effects of 40% induced haemorrhage on thermoregulation heart rate, haematological parameters, serum concentrations of proteins and sodium (Na) were investigated.

4.1 Thermoregulation and Heart Rate (HR)

In this study, the effects of 40% haemorrhage on physiological responses of goats were investigated. The rectal temperature (Tr) was influenced by haemorrhage (Fig.1); immediately post-haemorrhage, Tr value increased significantly and decreased at 6hrs. The initial rise in Tr could be related to a decrease in body-core to skin heat transfer due to hypovolaemia. Also, the retention of heat occurs due to an increase in peripheral resistance in response to haemorrhage [19]. The observed moderate hyperthermia could be associated with the calorigenic effect of hormones secreted post-haemorrhage. Catecho-lamines and adrenocorticotropic hormones assume a marked role in response to haemorrhage [9,4]. Immediate post-haemorrhagic hyperthermia has been reported in previous studies in goats [14,20,21]. The observed hypothermia which occurred over 6 hrs (Fig.1) may be due to decreased metabolism. The response to haemorrhage was associated with impaired cardiac output, lower metabolic rate and lower heat production in rats [22,23]. Similar observations have been reported in sheep[12] and rabbits [24].

The respiratory rate (RR) increased significantly in haemorrhaged animals (Fig.2). The rise in RR is likely to be associated with a decrease in oxygen supply. Blood loss and significant fall in PCV, Hb and erythrocyte count (Figs.4, 5, 6) resulted in anaemic hypoxia, thus stimulating chemoreceptors, the carotid and aortic bodies. Moore et al. [25] reported that the elevation of RR is a compensatory mechanism aimed to reduction of CO₂ retention and elimination of H⁺, leading to normalization of arterial blood pH. An increase in RR value after haemorrhage has been reported in goats[26, 14] and rabbits [24]. However, previous studies [27, 28] reported a decline in RR in rats subjected to 50 % bleeding.

The cardiovascular responses of the goats to acute haemorrhage resulted in a significant increase in heart rate (HR) (Fig.3). This probably relates to an increase in the activity of sympathetic response. The rise of HR is considered as a compensatory process to maintain tissue perfusion. It generates elevation of cardiac output and blood pressure [29]. Hypovolaemia promoted a strong sympathetic stimulation leading to an increase in HR in sheep [30], dogs and rabbit [31] and humans [5]. The reported increase in HR in haemorrhaged goats represented a compensatory change that assists in the recovery process.

4.2 Erythrocytic Parameters

In the present study, a loss of 40% of total blood volume resulted in significantly lower PCV values (Fig.4) associated with a significant decrease in Hb concentrations and erythrocyte count (Figs.5 and 6) after 6hrs. Immediately post haemorrhage, the values of PCV and RBC count were apparently normal because similar proportions were lost from plasma volume and erythrocytes. After acute haemorrhage, the mobilization of erythrocytes from storage organs such as the spleen, liver and pulmonary circulation occurs as an urgent compensatory process [32]. The subsequent decrease in PCV, Hb concentration and RBC count was presumably caused by shifting of water from the interstitial fluids to restore blood volume. Previous studies reported a progressive decline in PCV, Hb concentrations and RBC count after haemorrhage [14,12].

4.3 Leukocytic Parameters

Acute haemorrhage in goats resulted in a decrease in TLC immediately post-haemorrhage then a significant increase after 6 hrs (Fig. 7). The initial decline in TLC may be associated with haemodilution whereas the subsequent increase of TLC after 6hrs is probably attributed to splenic contraction. Activation of the immune system and inflammatory reactions have been reported as a first response of the body to haemorrhagic injuries [33]. Immature leukocytes appear in the blood and shift of neutrophils from the marginal pool and bone marrow to circulation occurs especially in severe haemorrhage [34]. A similar pattern of TLC has been reported by Abdalla and Abdelatif [14] and Argolo et al. [35] in goats and Sousa et al. [12] in sheep.

The results also indicate that acute haemorrhage caused significantly lower lymphocyte ratio associated with a higher ratio of neutrophils (Figs. 8 and 9). Lymphopenia may be attributed to the release of ACTH and cortisol in response to haemorrhage. Swenson [36] reported that
ACTH induces dissolution of lymphocytes and increased antibody concentration in the blood. Tyan [37] suggested that the decrease in peripheral blood lymphocytes in rats under haematopoietic stress is controlled by mechanisms intrinsic to the bone marrow itself. The increase in neutrophils ratio may be attributed to change in the haematopoietic microenvironment. Similarly, an immediate decline of TLC values and neutrophilia have been reported in goats subjected to 20% bleeding [20] and in dogs after haemorrhagic shock [38].

4.4 Serum Proteins

After haemorrhage, there was a decrease in serum total protein and albumin concentrations (Figs.12 and 13). This is clearly related to haemodilution. Furthermore, hypovolaemia induced drop in hydrostatic pressure causes a reduction in filtration fraction and an increase in lymphatic flow resulting in a net movement of interstitial fluid into the vascular space [39]. There was no significant change in value immediately post haemorrhage; the levels of PCV and total proteins are correlated weakly with hypovolaemia in acute bleeding because it takes several hours for fluid redistribution and for renin-angiotensin–aldosterone system to affect the PCV. (REFERENCES) Proteins may enter the circulation with water to prevent the dilutional decrease of protein concentration [40]. In the present study, total protein and albumin concentrations maintained lower levels compared to pre-bleeding values until the end of the experimental period. However, the current result contrasted the findings of Sousa et al. [12] in sheep. The authors observed a rapid return to baseline levels in proteins (24hrs); this was attributed to the existence of secondary circulation of proteins from the capillaries to the tissue fluids, which return to circulation via the lymph [41].

4.5 Serum Na Concentration

The results indicate that acute haemorrhage resulted in a decrease in serum Na concentration (Fig.14). This response could be attributed to haemodilution which involves entry of extravascular fluids into the vascular spaces [42]. Also, it could be related to an increase in circulating arginine vasopressin (AVP) that promotes tubular water absorption. Fall in Na concentration during haemorrhagic shock has been reported in rabbits revealing an influx of Na due to acidosis [24]. The current result agrees with Abdalla and Abdelatif [14] in goats subjected to 30% bleeding and with Wintour et al. [30] who reported a decrease in serum Na level in sheep subjected to 20% bleeding. On the other hand, Sharma et al. [2] observed hypernatraemia in rats exposed to haemorrhagic shock.

5. CONCLUSIONS

The studies provide basic information regarding thermoregulation and haematological responses of goats to acute blood loss caused by various factors which include traumatic haemorrhage and bleeding usually associated with surgical operations and parturition. The findings have implications in the fields of veterinary haematology and surgery. Also, they may be utilized in resuscitation and restoration of normal blood functions. The general patterns reported in the studies suggest that the goat can be adopted as a useful mammalian research model for investigations that would be valuable in comparative medicine.

ETHICAL APPROVAL

Ethical issues were addressed adequately according to veterinary and institutional guidelines.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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