

Thermoregulation and Metabolic Responses to Acute Haemorrhage in Adult Nubian Goats

ABSTRACT

The study was performed to investigate the effects of Acute haemorrhage on physiological responses to haemorrhage in goats. The effects 40% bleeding were evaluated in adult goats. The group subjected to haemorrhage had higher rectal temperature (Tr), respiration rate (RR) and heart rate (HR) compared to the control. The treated group had lower PCV, Hb concentration, erythrocyte count and TLC compared to the control. The ratios of lymphocytes, monocytes and eosinophils decreased, whereas the neutrophil ratio increased in treated group compared to the control. The treated groups had lower serum protein, albumin and lower serum Na concentrations compared to the control.

INTRODUCTION

Haemorrhagic shock is a major cause of morbidity and mortality in surgery and trauma due to ischaemic lesions followed by multiple organ dysfunction (Tintinalli, 2010). A compensatory redistribution of blood volume may occur from less noble tissues to more vital and survival organs (Kelley, 2005; Moore *et al.*, 2003).

Shock is an abnormal physiological state characterized by hypotension caused by the recruitment of extracellular fluids (Rhee *et al.*, 2003;

Sharma et al., 2012). Acute haemorrhage, a form of hypovolaemic shock. Bleeding of 15 to 20% is clinically detectable, while 30 to 40% bleeding causes life-threatening circulatory failure (Marino, 2007). Decreases in circulating blood volume during haemorrhage can depress cardiac output and severe haemorrhage impairs delivery of oxygen and nutrients to meet the metabolic needs of the tissues producing a state of shock (Gutierrez et al., 2004). This usually leads to the reduction of available oxygen to tissue cells, hypothermia, metabolic acidosis (Dutton, 2007; Abreu et al., 2010). and cardiac and respiratory arrest (Pacagnella et al., 2013).

The primary physiologic response to compensate for haemorrhagic shock is via sympathetic nervous system; baroreceptors in the carotid sinus and chemoreceptors sense hypovolaemia and hypoxaemia, respectively (Edwards, 2001). Also endocrine mediated mechanisms help to maintain perfusion in shock; the release of catecholamines activates α_1 receptors in peripheral vascular smooth muscle causing peripheral vasoconstriction (Miyagatani et al., 1999). Proportional increase in heart rate (HR) and decrease in mean arterial pressure (MAP) have been reported in animals exposed to haemorrhage (Rose et al., 1987; Douzinas et al., 2008; Sousa et al., 2012). The cause of death in animals subjected to haemorrhagic shock was cardiac arrhythmia (Eichstaedt et al., 2000).

Recently, studies on small animals have been conducted to obtain better evaluation of haemodynamic and metabolic parameters which occur during haemorrhagic shock. 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 new model, based on our previous study on haemorrhage (Abdalla and Abdelatif, 2008). Goats

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 acute haemorrhage in Nubian goats.

MATERIALS AND METHODS

Animals and Diet: The study used 6 female mature 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 during late winter at the Department of physiology.

Experimental Design: For all animals, the baseline 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. The acute phase responses to haemorrhagic shock were monitored for three days.

Thermoregulation and heart rate (HR) measurements: The measurements of rectal temperature (Tr) of the goats were made to the nearest $\pm 0.1^{\circ}\text{C}$ 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.

Blood volume and blood sampling: The total blood volume was measured utilizing plasma volume determined by Evans blue dye (Pirkle and Gann, 1976). Blood samples were taken before 1 day, before and immediately after bleeding and 6, 24, 48, 72 hrs post bleeding. Blood samples were collected using 5 ml disposable syringes. 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.

Blood 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 (Kelly, 1984; Jain, 1993).

Serum analysis: The concentrations of serum total protein, albumin, were determined by colorimetric methods using kits (Spinreact, S.A. Spain). The concentration of sodium (Na) was determined by flame photometer technique (Wootton, 1974).

Statistical analysis: The experimental data were subjected to appropriate analysis of one way anova using the SAS package (1998). The data were presented as mean ± standard deviation (SD).

RESULTS

Rectal temperature (Tr): The effect of haemorrhagic shock on Tr is shown in Fig.1. The initial values of Tr in experimental animal was 38.8 ± 0.22 °C. For all animals (Tr) showed significant ($P < 0.01$) increase immediately after haemorrhagic shock. Thereafter, the animal showed progressive decline in (Tr) until 2hr and then showed slight elevation that was maintained until 24hr.

Respiration rate(RR): Fig.2 show that the initial mean values of RR for experimental animal was 28 ± 4.66 breaths/min. The animal 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, 6hrs after haemorrhagic shock. The RR values returned to normal after 24hrs in goats.

Heart rate (HR): Fig.3 show that the initial mean values of (HR) in animals was 76 ± 6.32 beat/min. There was a significant ($P < 0.01$) increase in HR immediately following bleeding and at 2,3,4,5,6hr after haemorrhagic shock. The mean HR values returned to normal after 24hrs in all experimental goats.

Packed cell volume(PCV): The responses of Packed cell volume(PCV) are shown in Fig.4. The initial mean values was $29.4 \pm 2.51\%$. Immediately following haemorrhagic shock ,there was no significant change in PCV level in experimental animals. Then, the animal showed progressive and significant ($P < 0.001$) decline in PCV values at 6,24,48 and 72hrs.

Haemoglobin concentration(Hb): Fig.5 shows that the initial pre-haemorrhagic shock values of Hb was 11.7 ± 1.28 g/dL. The general pattern of Hb values presented in Fig. 5 indicates significantly ($P < 0.001$) decrease

immediately post haemorrhagic shock and at 6, 24,48,and 72hrs post-haemorrhagic shock.

Erythrocyte count: Fig.6 indicates that the initial values of erythrocyte count was $12.3 \times 10^6 \pm 1.58 /\mu\text{L}$. No significant change immediately following haemorrhagic shock. In experimental animals, erythrocytic count decreased significantly ($P<0.05$) at 6,48 and72hrs and significantly($P<0.01$) at 24hrs post-haemorrhagic shock.

Total leukocyte count (TLC):The effect of haemorrhagic shock on TLC are shown in Fig. 7. The experimental group had initial pre-bleeding values of about $9.6 \times 10^3 \pm 1.78 /\mu\text{L}$. There was significant decrease ($P<0.01$) in TLC values immediately post-haemorrhagic shock. Thereafter, experimental animals showed significant ($P<0.05$) increase in TLC at 6 and 24hrs, then re-established normal values of TLC After 48hrs.

Differential leukocyte count (DLC): Figs.8,9,10and 11 show the effect of haemorrhagic shock on lymphocyte, neutrophil, monocyte and esinophil ratios, respectively. The lymphocyte ratio was significantly ($P<0.01$) lower compared to pre-bleeding values at 6,24,48 and 72hrs post-bleeding (Fig.8), while the neutrophil ratio was increased significantly ($P<0.01$) compared to pre-bleeding values at 6,24hrs and increased significantly ($P<0.05$) at 48 and 72hrs post haemorrhagic shock(Fig.9). The monocyte ratio decreased immediately after bleeding ,lower values were maintained at 72hrs post-haemorrhagic shock (Fig.10).The eosinophil ratio decreased significantly ($P<0.05$) at 6 and 24hrs post -bleeding, then increased to return normal values at 72hrs(Fig.11).

Serum total protein: Fig .12 show that the initial total protein mean values was $7.05 \pm 0.68\text{g/dL}$. The values reported in Figs show that there was decrease in total protein levels. The decline was significantly ($P<0.01$) at 6, 24,48 and 72hrs to attain 4.6 g/dL at 72hrs post-bleeding.

Serum albumin: The effect of acute haemorrhage on serum albumin concentration are shown in Fig.13. The initial values for experimental animals was $3.1\pm 0.33\text{g/dL}$. Albumin concentrations decreased significantly ($P<0.05$) at 6 and 48hrs post-haemorrhagic shock and maintained lower level compared to pre-bleeding values until the end of experimental period.

Serum sodum(Na): Fig.14 show the effect of haemorrhage on Na concentrations. The initial Na mean values was $128\pm 1.92\text{ mEqL}^{-1}$. In experimental animal, The values declined significantly ($P<0.05$) at 6, 24,48 and 72hrs postbleeding.

DISCUSSION

In this study, the effects of acute 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 ($P< 0.05$) and decreased at 6hrs. The initial rise in Tr could be related to decrease in body-core to skin heat transfer due to hypovolaemia. Also, retention of heat occurs due to an increase in peripheral resistance in response to haemorrhage (Vanter, 1974). The observed moderate hyperthermia could be associated with the calonegic effect of hormone secreted post-haemorrhage. Catecholamines

and adrenocorticotrophic hormones assume marked role in response to haemorrhage (Miyagatani *et al.*,1999; Gutierrez *et al.*,2004). Immediate post-haemorrhagic hyperthermia has been reported in previous studies in goats(Abdalla and Abdelatif, 2008, Abdalla and Abdelatif, 2010; Abdelatif and Abdalla, 2009) .The observed hypothermia which occurred over 6 hrs (Fig.1) may be due to decreased blood cell metabolism. This response was associated with impaired cardiac output, lower metabolic rate and lower heat production in rats (Brown *et al.*, 2005; Henderson *et al.*, 2000). Similar observations have been reported in sheep(Sousa *et al.*, 2012), rabbits (Fontelles *et al.*,2007).

The respiratory rate (RR) increased significantly in haemorrhaged animals (Fig.2).The rise in RR is likely to be associated with 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 carotids and aortic bodies. Moore *et al.* (2003) 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 values after haemorrhage has been reported in goats:(Maltz *et al.*, 1984; Abdalla and Abdelatif, 2008) and rabbits (Fontelles *et al.*,2007). However,(Jochem, 2001, Jochem *et al.*, 2001) reported 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 compensatory process to maintain tissue perfusion. It generates elevation of cardiac output and blood pressure (Guyton, 2002).

Hypovolaemia promoted a strong sympathetic stimulation leading to increase in HR in sheep (Wintour et al.,1995), dogs and rabbit (Schadt and Ludbrook, 1991), horses (Malikids et al.,1991), and humans (Dutton,2007). The reported increase in HR in haemorrhaged goats represented a compensatory change that assists in the recovery process.

In present study, loss of 40% of total blood volume resulted in significantly lower PCV values (Fig.4) associated with significant decrease in Hb concentrations and erythrocyte count (Fig.5 and 6) after 6hrs. Immediately posthaemorrhage, 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 occur as an urgent compensatory process (Reece,1993). 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 progressive decline in PCV, Hb concentrations and RBC count after haemorrhage (Abdalla and Abdellatif, 2008; Sousa et al., 2012; Vnuk et al., 2009 and Matot et al., 2008).

Acute haemorrhage in goats resulted in 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. An activation of the immune system and inflammatory reactions have been reported as a first response of the body to haemorrhagic injuries (Maier,2000). Immature leukocytes appear in the blood and shift of neutrophils from marginal pool and bone marrow to circulation occurs

especially in severe haemorrhage (Duncan et al.,1994). A similar pattern of TLC has been reported by Argolo et al(2018) and Abdalla and Abdelatif (2008) in goats and Sousa et al.(2012) in sheep.

The results also indicate that acute haemorrhage caused significantly lower lymphocyte ratio associated with higher ratio of neutrophils (Figs. 8 and 9). Lymphopenia may be attributed to release of ACTH and cortisol in response to haemorrhage. Swenson (1993) reported that ACTH induces dissolution of lymphocytes and increased antibody concentration in the blood. Tyan (1982) 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 haematopoietic microenvironment. Similarly, an immediate decline of TLC values and neutrophilia has been reported in goats subjected to 20% bleeding (Abdelatif and abdalla, 2009) and in dogs after haemorrhagic shock (Shatney et al., 1981)

After haemorrhage, there was a decrease in serum total protein and albumin concentrations (Figs.12 and 13). This is clearly related to haemodilution. The decrease in total protein was shown to be a consequence of haemorrhage and the loss of albumin is faster than other proteins because of its small size (Kerr, 2003). There was no significant change in value immediately posthaemorrhage; 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 haematocrit. Proteins may enter the circulation with water to prevent dilutional decrease of protein concentration (Block et al.,1989). In the present study, total protein and albumin concentrations

maintained lower level compared to pre-bleeding values until the end of experimental period. This result partially agrees with Milikides et al.(1991) who reported return of albumin to initial levels 8 days post-haemorrhage in horses. However , the current result contrasted the findings of Sousa et al.(2012) in sheep. The authors observed rapid return to baseline levels in proteins (24hrs) and attributed that to the existence of secondary circulation of proteins from the capillaries to the tissue fluids, which return to circulation via the lymph (Thrall et al., 2006).

The current 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 (Hjelmqvist et al.,1991) . Also it could be related to increase in circulating arginin vasoptrssin (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 (Fontelles et al., 2007). The current result agrees with Abdalla and abdelatif (2008) in goat subjected to 30% bleeding and with Wintour et al.(1995) who reported a decrease in Na level in sheep subjected to 20% bleeding. On the other hand, Sharma et al.(2012) observed hypernatraemia in rats exposed to haemorrhagic shock .

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UNDER PEER REVIEW

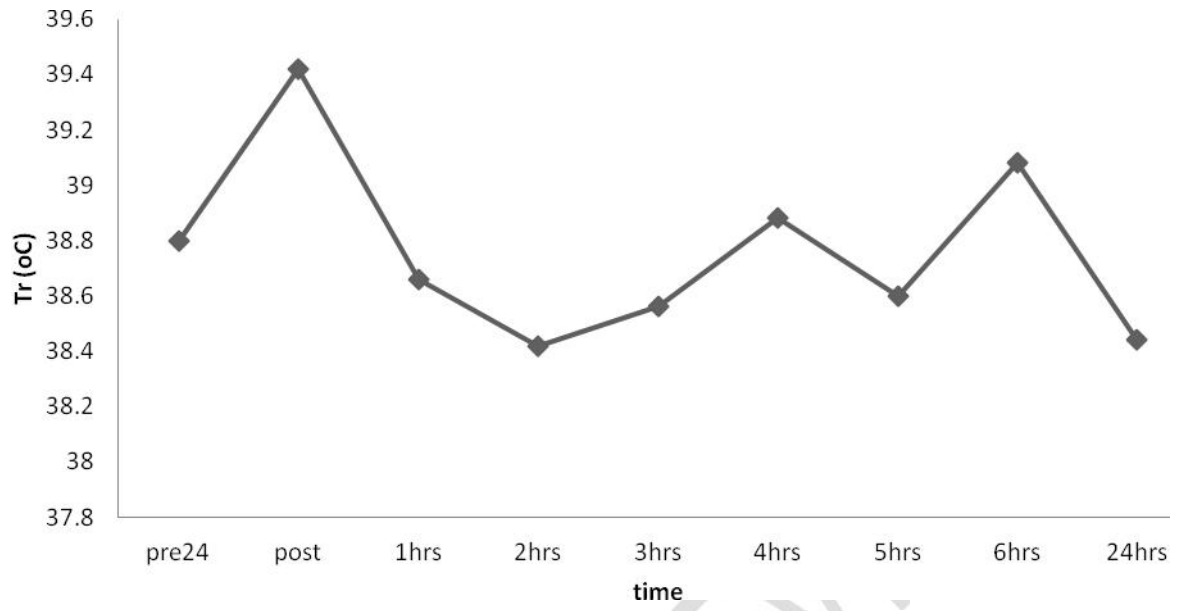


Fig.1 Effect of acute haemorrhage on rectal temperature (Tr) in adult Nubian goats.

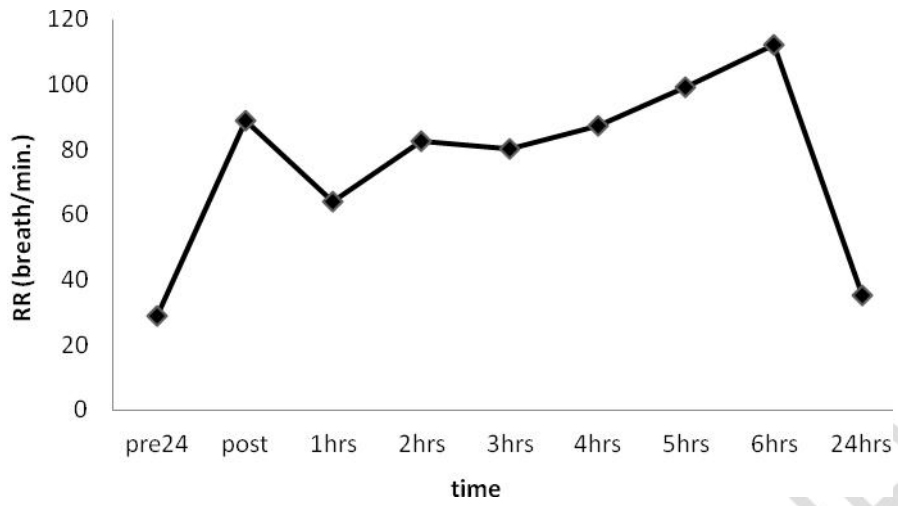


Fig.2 Effect of acute haemorrhage on respiratory rate (RR) in adult Nubian goats.

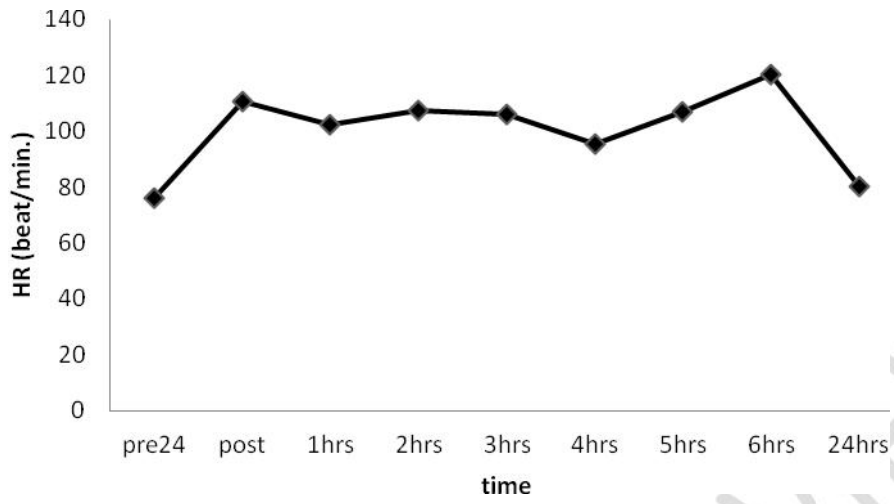


Fig.3 Effect of acute haemorrhage on heart rate (HR) in adult Nubian goats.

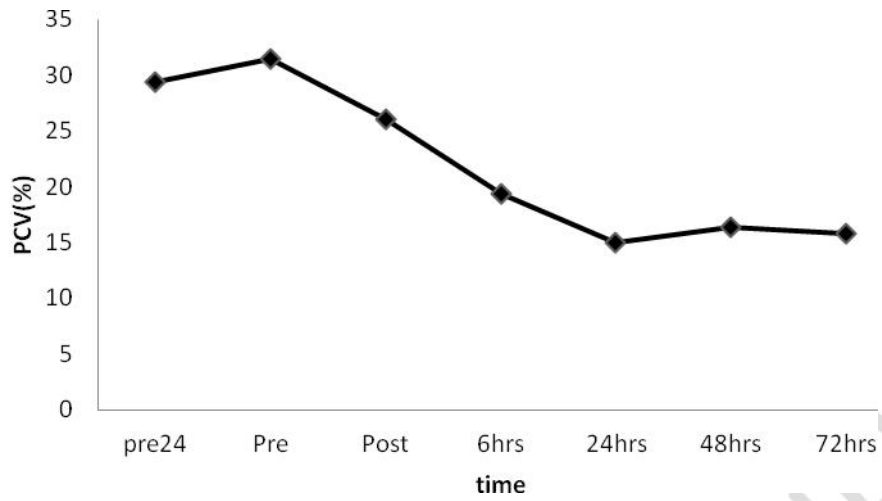


Fig.4 Effect of acute haemorrhage on packed cell volume (PCV) in adult Nubian goats.

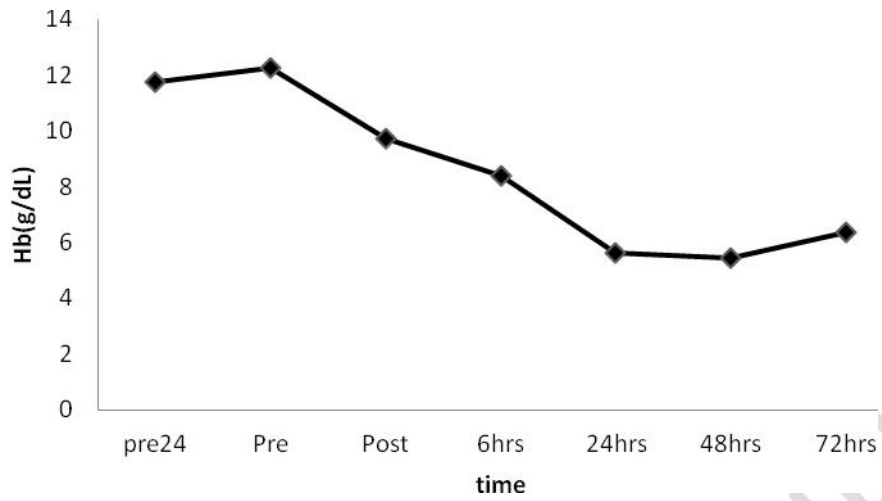


Fig.5 Effect of acute haemorrhage on haemoglobin concentration(Hb) in adult Nubian goats.

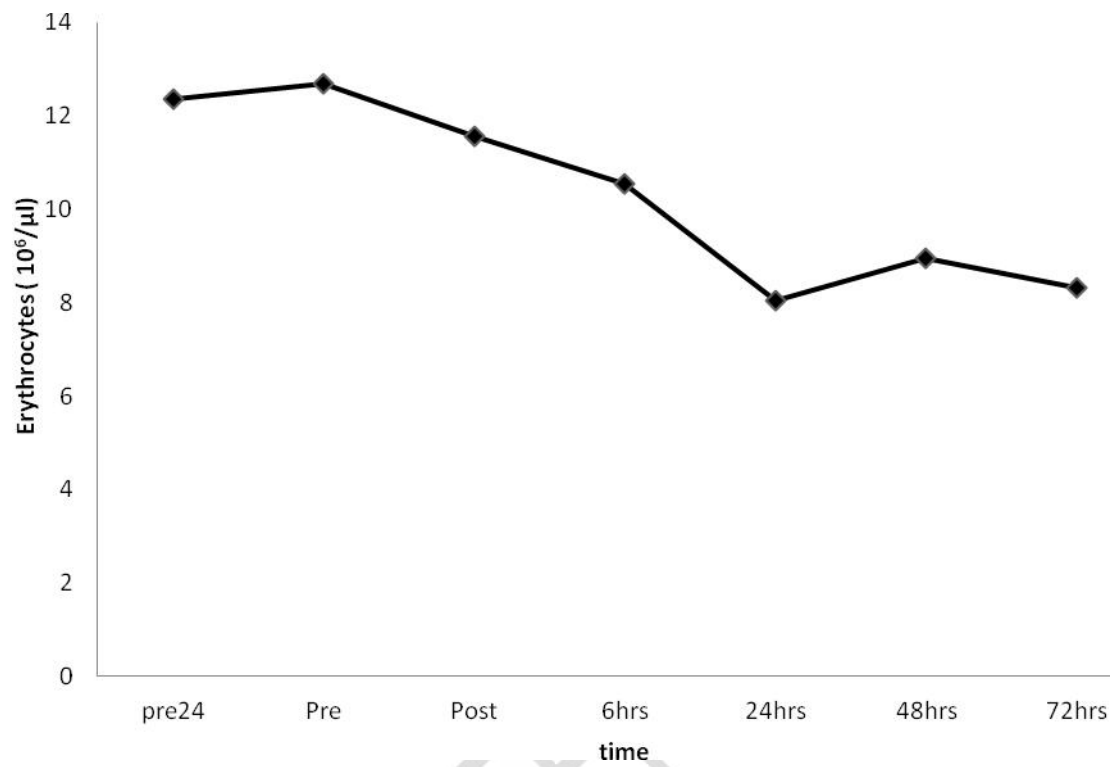


Fig.6 Effect of acute haemorrhage on erythrocytes count in adult Nubian goats.

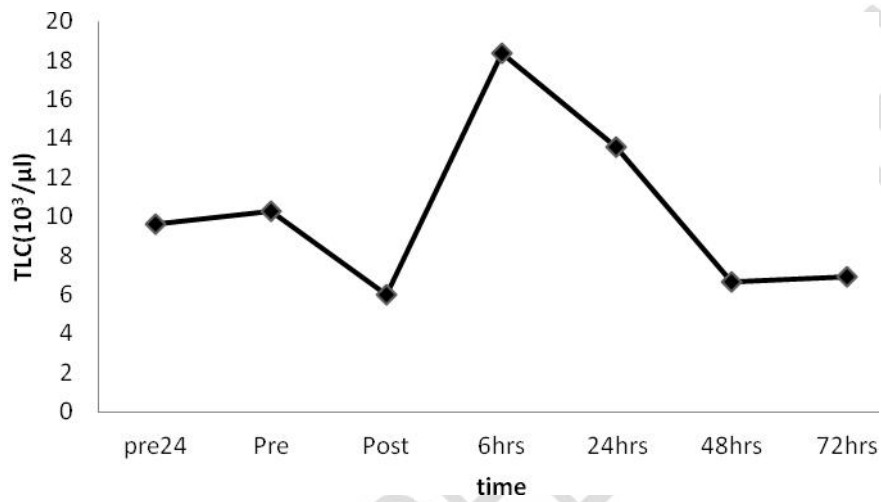


Fig.7 Effect of acute haemorrhage total leukocyte count (TLC) in adult Nubian goats.

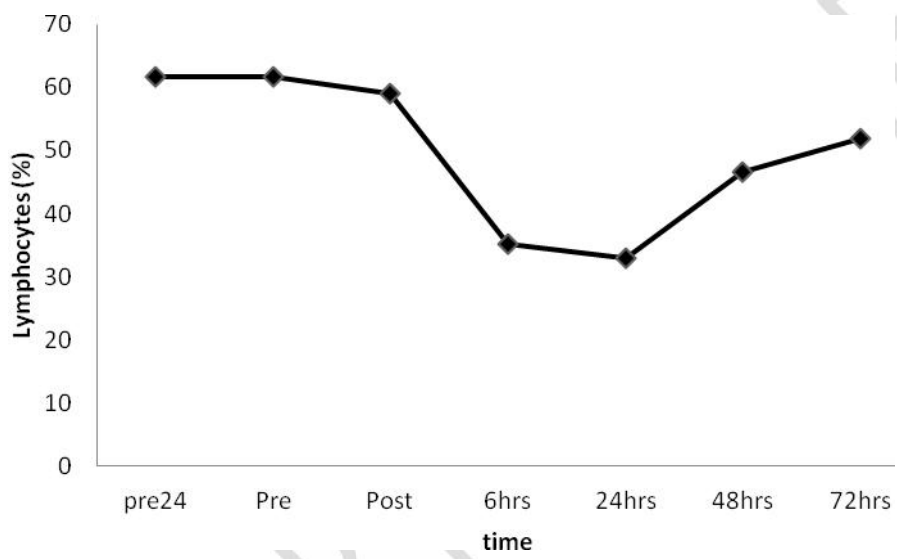


Fig.8 Effect of acute haemorrhage on lymphocyte ratios in adult Nubian goats.

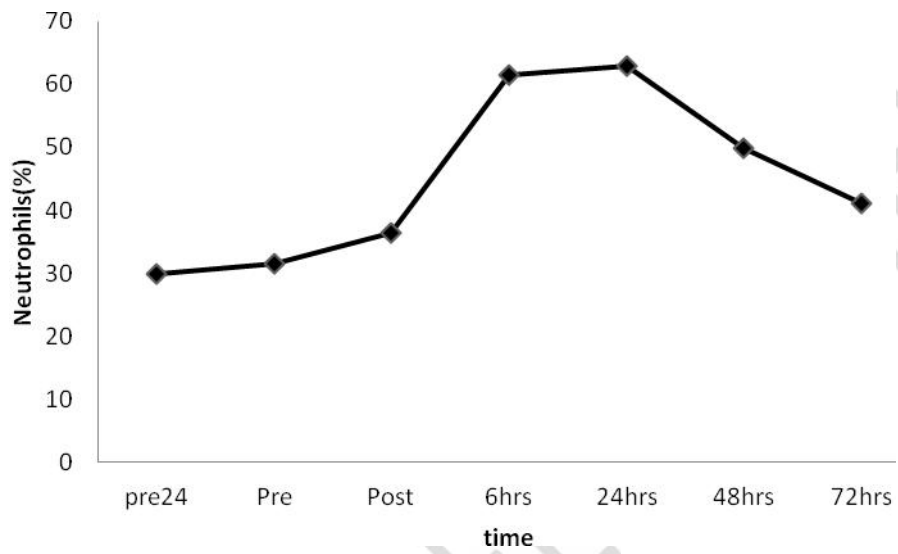


Fig.9 Effect of acute haemorrhage on neutrophil ratios in adult Nubian goats.

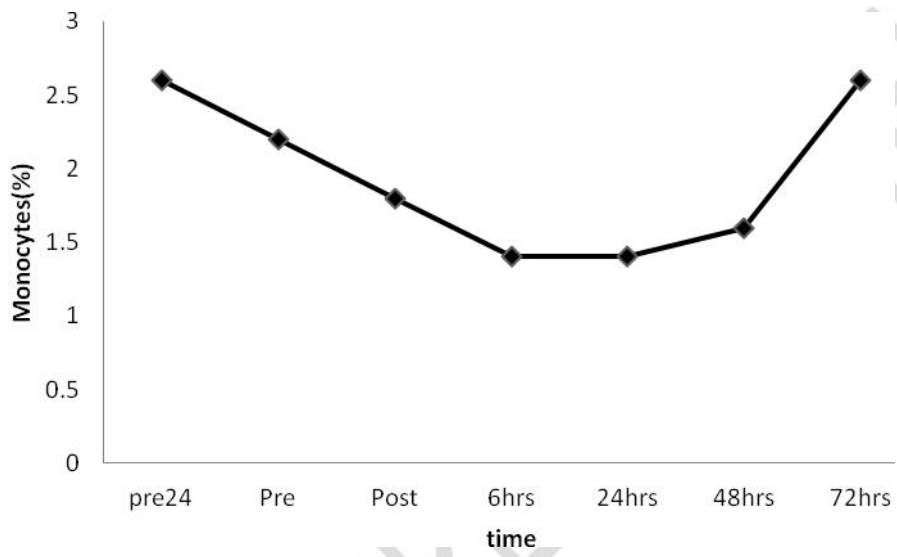


Fig. 10 Effect of acute haemorrhage on monocyte ratios in adult Nubian goats.

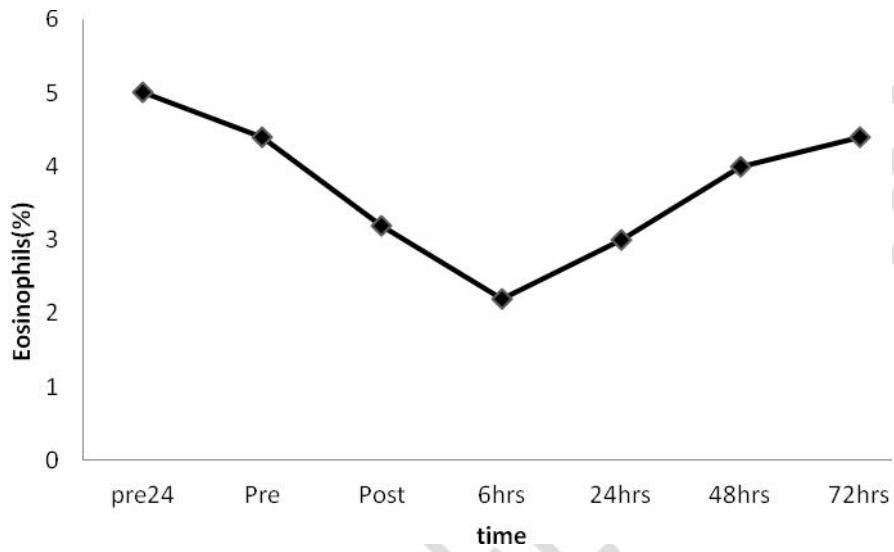


Fig. 11 Effect of acute haemorrhage on eosinophil ratios in adult Nubian goats.

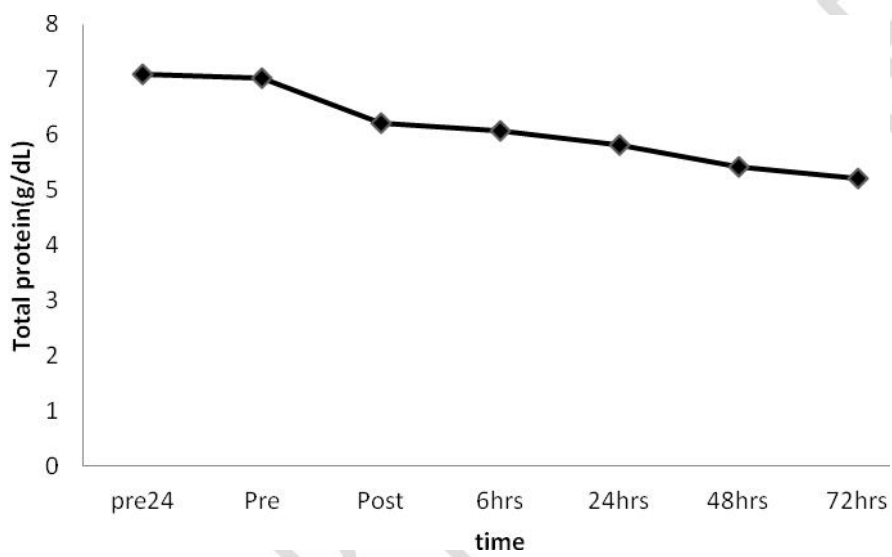


Fig. 12 Effect of acute haemorrhage on total protein concentration in adult Nubian goats.

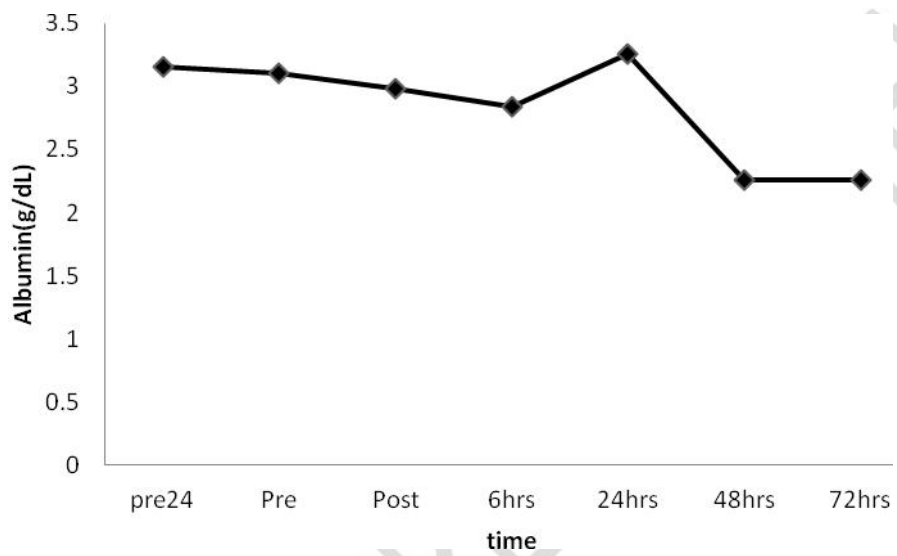


Fig. 13 Effect of acute haemorrhage on albumin concentration in adult Nubian goats.

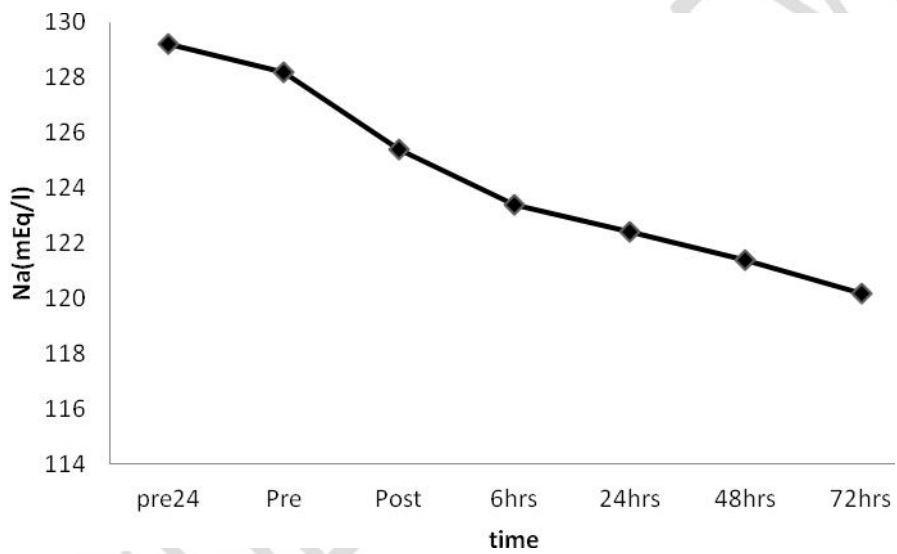


Fig. 14 Effect of acute haemorrhage on sodium concentration (Na) in adult Nubian goats.

Parameters	Pre24hrs	Post-bleeding	1hr	2hrs	3hrs	4hrs	5hrs	6hrs	24hrs
Tr (⁰ C)	38.80 ±0.22	39.10 ±0.16	37.9 ±0.40	37.96 ±0.16	38.00 ±0.15	38.34 ±0.30	38.50 ±0.33	38.66 ±0.32	38.72 ±0.26
RR (breath/min.)	28.80 ±4.66	79.20 ±5.77	74.00 ±9.50	82.00 ±6.63	79.60 ±7.80	86.40 ±8.70	98.80 ±2.68	112.20 ±5.22	35.20 ±3.70
HR (beat/min.)	67.00 ±8.0	100.40 ±8.41	125.00 ±7.68	118.80 ±8.90	116.00 ±8.00	109.00 ±7.40	111.20 ±10.44	120.20 ±12.26	80.00 ±11.04

Parameters	Pre24hrs	Pre-bleeding	Post-bleeding	6hrs	24hrs	48hrs	72hrs
PCV(%)	29.40 ±2.51	31.40 ±3.91	27.40 ±3.65	19.41 ±3.5	15.00 ±2.35	16.31 2.30±	15.80 ±2.17
Hb(g/dL)	11.74 ±1.28	12.26 ±1.27	11.12 ±0.98	8.40 ±1.80	5.60 ±0.72	5.44 ±0.93	6.36 ±0.43
RBCs(x10 ⁶ /μl)	12.34 ±1.58	12.69 ±1.74	11.54 ±0.75	10.55 ±0.99	7.86 ±0.52	8.32 ±0.42	8.67 ±0.52

Parameters	Pre24hrs	Pre-bleeding	Post-bleeding	6hrs	24hrs	48hrs	72hrs
TLC($\times 10^3 /\mu\text{l}$)	9.6 ± 1.78	10.30 ± 1.84	6.00 ± 1.02	18.36 ± 6.64	13.58 ± 2.84	6.62 ± 1.18	6.69 ± 1.81
Lymphocytes (%)	61.6 ± 1.16	61.6 ± 3.20	59.00 ± 6.20	35.20 ± 6.53	33.00 ± 7.90	47.00 ± 4.00	51.00 ± 6.84
Neutrophils (%)	30.00 ± 2.55	31.60 ± 2.70	36.40 ± 4.39	60.40 ± 6.62	62.80 ± 7.73	47.80 ± 7.79	41.20 ± 4.77
Monocytes (%)	4.00 ± 1.00	4.00 ± 1.23	2.80 ± 1.30	1.40 ± 0.55	1.40 ± 0.09	1.80 ± 0.83	2.80 ± 0.80
Eosinophils (%)	4.8 ± 0.84	4.40 ± 1.40	3.60 ± 1.51	2.80 ± 1.3	3.20 ± 1.64	4.00 ± 1.35	4.40 ± 1.67

Parameters	Pre24hrs	Pre-bleeding	Post-bleeding	6hr	24hr	48hr	72hr
TP(g/dL)	6.52 ± 0.68	6.98 ± 1.12	5.84 ± 1.00	5.18 ± 1.09	4.70 ± 1.18	4.68 ± 0.74	5.04 ± 0.29
Alb(g/dL)	3.30 ± 0.33	3.46 ± 0.29	2.70 ± 0.30	2.52 ± 0.41	2.80 ± 0.30	2.74 ± 0.30	2.90 ± 0.39
Na(mEq/L)	129.20 ± 1.92	129.60 ± 1.52	125.40 ± 2.70	123.40 ± 2.70	123.20 ± 2.59	123.40 ± 2.40	126.00 ± 2.55