Physiological Responses to Trauma-Haemorrhage (T/H) in Relation to Gender (Sex) in Adult Nubian Goats (*Capra hircus*)

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Original Research Article

ABSTRACT

Aims: The effects of gender and trauma-haemorrhage (T/H) on thermoregulation and blood constituents were investigated in adult Nubian goats.
Methodology: Twenty adult goats (10 males and 10 females) were used in the experiment. For each gender, animals were randomly assigned to two groups of 5 animals each, a control group and a treated group that was subjected to surgical soft tissue trauma (laparotomy) and 30% bleeding of calculated total blood volume. Thermoregulation, BW, haematological and serum biochemical parameters were monitored for 7 weeks.
Results: The results showed significant increases in RR ($p<0.05$) and HR ($p<0.001$) following T/H in treated groups. The females had significantly lower rectal temperature Tr ($p<0.05$) and higher heart rate HR ($p<0.001$) values in response to T/H. After T-H, the PCV and Hb concentration significantly

(p<0.001) decreased in both treated groups until week 2, both parameters were lower (p<0.001) in females compared with males. The interaction between gender and T/H indicated lower (p<0.01) Hb concentration in the female group. The TLC values was increased significantly (p<0.001) in the female treated group after 6 hrs and at day 4 in the male treated group. The interaction between gender and T/H indicated higher (p<0.001) TLC in the female treated group. The ratios of lymphocytes decreased (p<0.001) and neutrophils increased (p<0.001) in treated groups compared with control groups until day 6. The interaction effects between gender and T/H indicated a lower (p<0.01) lymphocyte ratio and a higher (p<0.01) neutrophil ratio in the female treated group. Serum concentrations of total protein (p<0.001) and albumin (p<0.01) were significantly lower in T/H goats reflecting haemodilution and tissue catabolism. Following T/H, serum urea (p<0.01) and plasma glucose (p<0.001) concentrations were significantly higher in treated groups until day 2; and the females had significantly (p<0.001) higher glucose concentration compared with males.

Conclusions: Gender had marked effects on the responses to T/H. Female goats subjected to T/H had higher physiological responses compared to male treated goats.

1. INTRODUCTION

Trauma is one of the leading causes of death in the world [1] as it could be associated with the development of multisystem organ dysfunction/failure and sepsis [2]. Studies on humans, as well as animal models, indicate a gender specific responsiveness of the physiology and organ systems with regard to trauma and haemorrhage, trauma, and sepsis [3,4]. Studies also indicated that male gender and age are risk factors for the development of sepsis, multiple organ failure and mortality following trauma and T/H [5-7].

The investigations on gender specific differences in the response to traumatic injury in animal models suggested that the dimorphic response is partially based on the levels of the steroid hormones oestrogen and testosterone, or their derivatives [8-10]. Studies have indicated that male sex steroids appear to play a deleterious role in the development of organ dysfunction after T/H, whereas female sex hormones may have protective effects [11-13]. Specifically, studies indicated that androgens are responsible for immune and cardiac muscle depression after T/H in males [14]. In contrast, female sex steroids seem to exhibit immune and cardiac protective properties after trauma and severe blood loss in mice [15], rats [16] and humans [17]. Wichmann et al. [18] reported significant gender differences in B lymphocyte, T lymphocyte and natural killer (NK) cell counts following surgery, with men showing reductions in cell numbers for up to 5 days.

Trauma and haemorrhage are known to induce myocardial dysfunction, decreasing cardiac output and blood flow [19]. This effect is more pronounced in male mice. In contrast, proestrus females have shown better regulation of cardiac function and blood volumes following T/H when compared to males, with significant improvements in cardiac output and performance as well as increased circulating blood volume [20]. Mizushima et al. [21] indicated that the left ventricular performance, cardiac output, heart rate and hepatic function decreased significantly 24 hours after T/H in male rats. Researchers reported that the administration of oestradiol restores the functions of the organs after T/H in male mice [22] and rats [23].

The effects of gender on T/H have been previously studied in laboratory animal species and humans, but investigations on small ruminants have not been reported. This experiment was performed to explore the effects of gender on the physiological responses to T/H in adults Nubian goats.

2. MATERIALS AND METHODS

2.1 Experimental Animals, Housing and Management

Twenty mature, apparently healthy goats, obtained from the local market, were used in the experiment. The goats were kept in the small ruminant unit at the Department of Physiology, Faculty of Veterinary Medicine, University of Khartoum. Animals were examined clinically and were given prophylactic treatments of

anthelmintic and antibacterial. The goats were maintained on a diet of dry lucerne hay (*Medicago sativa*) and tap water *ad libitum*. They were kept for an adaptation period of 2 weeks before experimentation so that they were accustomed to the experimental conditions and collection of blood samples. The experiment was conducted during January –February, 2018 (maximum temperature 37ºC, minimum 9.0ºC; mean relative humidity 30%).

2.2 Experimental Design

A total group of 20 adult goats (10 males and 10 females) were used in the experiment. For each gender, animals were randomly assigned to two groups of 5 animals each, a control group and a treated group that was subjected to surgical soft tissue trauma (laparotomy) and 30% bleeding of calculated total blood volume. For all groups of animals, the initial baseline physiological data were determined. Blood samples were collected pre-T/H, and post-T/H, at 6 hrs, and at 1, 2, 4, 6, 8 days. Thereafter, blood samples were collected weekly for 6 weeks. Thermoregulation, BW and haematological and serum biochemical parameters were monitored for 7 weeks.

2.3 Trauma and Haemorrhage Procedure

2.3.1 Laparotomy

The laparotomy was done according to a standard procedure [24]. Animals were fasted overnight before surgery. The left flank region of each goat was prepared for surgery by clipping and shaving the hair on the proposed surgical site; the site was scrubbed with povidone iodine 10% topical solution. Local anaesthesia, 2% w/v LidocaineHCl injection (PSI, Jeddah, Saudi Arabia) 10 ml were used. The animal was laid on its right side and a 15 cm mid- line incision was made in the left flank region. The laparotomy incision was closed in three layers from within outward; muscle layers and the subcutaneous layer were closed using chromic catgut size 2/0. The skin was closed using surgical sutures silk. Antimicrobial Almox L.A 15% injectable suspension (Star Laboratories, Pakistan) and anti-inflammatory Dexaphan (Pharma Swede, Egypt) medications were used for 5 days after surgery.

2.3.2 Bleeding

The induction of haemorrhage was performed in goats by withdrawal of 30% of total blood volume during the last stage of laparotomy. Graduated blood collection bags (JMS PTE Ltd, Singapore) were used for collection of 30% of calculated blood volume from the jugular vein. The total blood volume for each goat was calcaluted as 7.4% of body weight [25].

Blood volume (BV) = Body weight x 7.4/100
30% of total blood volume = BV X 30/100

2.4 Physiological Investigations

2.4.1 Rectal temperature (T$_r$)

The rectal temperature (T$_r$) of goats was measured by a digital clinical thermometer (Hartman-United Kingdom). The tip of the thermometer was inserted to a depth of approximately 4 cm into the rectum, and T$_r$ was measured with an accuracy of $\pm$ 0.1°C.

2.4.2 Respiratary rate (RR)

The RR was measured by visually counting the flank movements for one minute using a stopwatch. The measurement was done when the animal was standing quietly and breathing regularly.

2.4.3 Haert rate (HR)

The HR was obtained by monitoring the heart sounds for one minute using a stethoscope and a stopwatch. The measurement was done when the animal was standing quietly.

2.4.4 Body weight (BW)

During the experiments, the animals were weighed using a traditional balance (Kinlee - Hanging scale, China).

2.4.5 Erythrocytic and leukocytes parameters

The standard methods described in Essentials of Veterinary Haematology [26] were used for the determination of the erythrocytic indices and leukogram parameters.

2.4.6 Biochemical parameters

Serum total protein concentration was determined by Biuret method [27] using a kit (BioSystems, S.A., Spain). Serum albumin concentration was determined by the colorimetric method of Bromocresol green [28] using a kit (Bio Systems, S. A., Spain). Serum urea

concentration was determined by the enzymatic-colorimetric test (Berthlot) [29] using a kit (BioSystems, S.A., Spain). Serum bilirubin total and direct concentration were determined using a kit (Spinreact, S. A. Spain) based on the DMSO-colorimetric test [30]. The plasma glucose concentration was determined by the enzymatic colorimetric method [31] using a kit (Spinreact, S.A., Spain).

2.5 Statistical Analysis

The data collected were subjected to standard methods of statistical analysis using Statistical Package for the Social Sciences (SPSS, version 20) [32]. Analysis of variance (ANOVA), one-way test was used to examine the effect of gender on physiological response to T/H. Then, two-way ANOVA test was used to analyze the interaction between gender and T/H in adult goats. The results of physiological responses of male and female goats to T/H have been depicted in graphs and histogram.

3. RESULTS

3.1 Rectal Temperature (T_r)

Post-T/H, the treated female group had significantly ($p \leq 0.001$) lower T_r compared with other groups. At 6 hrs, both T/H groups had lower T_r compared to respective control groups, and female treated group had significantly ($p \leq 0.01$) lower T_r compared with control groups. Both female groups had significantly ($p \leq 0.05$) lower T_r compared with male control, at days 1 and 2. At days 4 and 6, both female groups had significantly ($p \leq 0.05$) lower T_r compared to male treated group. The male treated group had significantly ($p \leq 0.01$) higher T_r compared to the other groups, at day 8. At week 5, both female groups had significantly ($p \leq 0.05$) higher T_r compared to male control group (Fig. 1). At post-T/H, days 2 and 8, there were significant ($p \leq 0.05$) interactions between gender and T/H on T_r. The interaction indicated lower T_r in female group.

3.2 Respiratory Rate (RR)

Post-T/H, the female treated group had significantly ($p \leq 0.05$) higher RR compared with control groups. The female treated group had lower RR values from day 1 until day 4, while the male treated group had lower RR from day 2 until day 6 compared with respective control (Fig. 2).

3.3 Heart Rate (HR)

The general trend indicated an increase in HR after T/H. Post- T/H and at 6 hrs, both treated groups had higher HR compared with control groups, but the female treated group had significantly ($p \leq 0.01$) higher HR compared with control groups. The groups subjected to T/H had significantly ($P \leq 0.001$) higher HR value compared with the control groups at days 1 and 2. At day 2, the female treated group had significantly ($p \leq 0.001$) higher HR compared with male treated group. The female treated group had significantly ($p \leq 0.01$) higher HR compared with the other groups at days 4 and 8. At day 6, treated female group had significantly ($p \leq 0.001$) higher HR compared to the values obtained for the control groups. At week 2, female treated group had significantly ($p \leq 0.05$) higher HR compared with male groups. The female groups had significantly ($p \leq 0.05$) higher HR compared with values obtained for the male control group at week 4. At weeks 5 and 6, the female groups had significantly ($p \leq 0.001$) higher HR values compared to male groups (Fig. 3). There was significant ($p \leq 0.05$) interaction between T/H and gender on HR response at day 2 (Fig. 3). The interaction indicated higher HR in female group.

3.4 Body Weight (BW)

The initial values of BW showed a significant ($p \leq 0.001$) difference between male and female groups (32.8 and 22.7 kg, respectively). The male treated group had lower and female treated group had higher BW mean values compared with respective controls at weeks 2, 4 and 7. After T/H, treated groups revealed a decrease in BW mean values compared with control groups, and then the BW values increased in all experimental groups (Fig. 4).

3.5 Packed Cell Volume (PCV)

Generally, the treated groups maintained lower mean values of PCV compared with the control groups during the experimental period. The female treated group had significantly lower PCV value compared with control groups at day 1 ($p \leq 0.05$) and day 4 ($p \leq 0.01$). At day 2, the female treated group had significantly ($p < 0.01$) lower PCV value compared with the value obtained for other groups. From day 6 until week 6, the treated groups had significantly ($p \leq 0.001$) lower PCV values compared with the control groups. The normal values of PCV were recovered after 4 weeks in male treated group and 5 weeks in female treated group (Fig. 5).
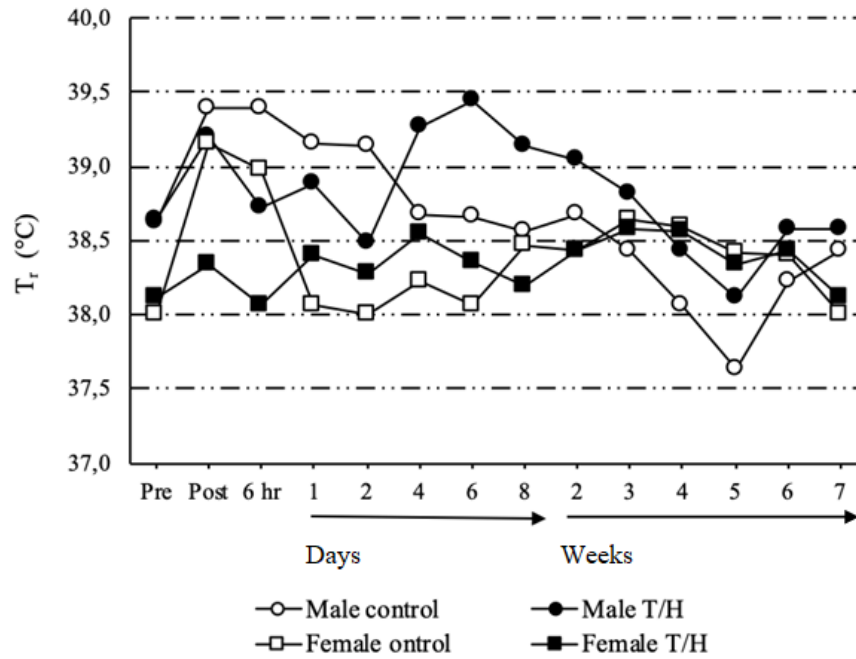
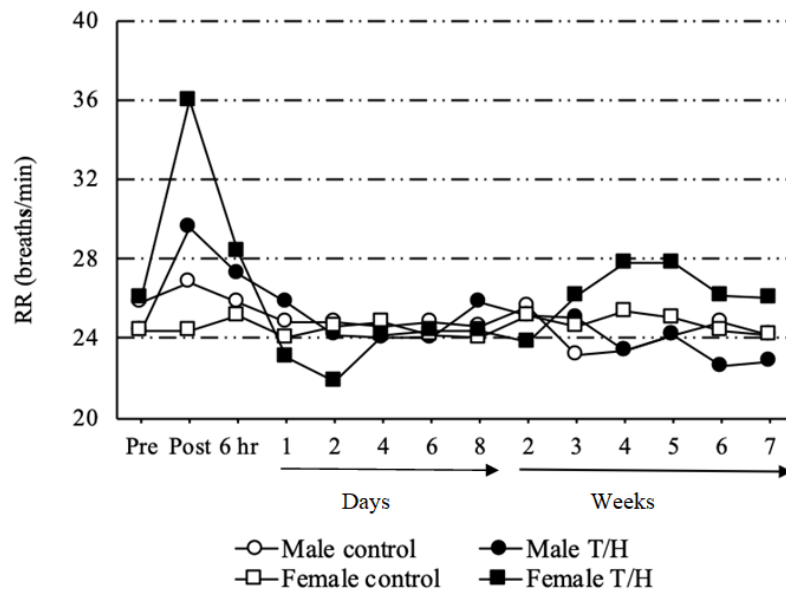Fig.1. Effects of gender and trauma/haemorrhage (T/H) on rectal temperature, T_r in adult goats.

Fig.2. Effects of gender and trauma/haemorrhage (T/H) on respiratory rate, RR in adult goats.

3.6 Haemoglobin (Hb) Concentration

At 6 hrs, both treated groups had lower Hb concentration compared with control groups. The treated male group had significantly ($p \leq 0.05$) lower Hb concentration compared with control groups. At day 1, the treated female group had significantly ($p \leq 0.01$) lower Hb concentration compared with other groups. The values of Hb were significantly ($p \leq 0.001$) lower in goats subjected to T/H compared with the control groups; also the female treated group had a significantly ($p \leq 0.001$) lower Hb concentration compared with male

treated group at days 2, 4 and 6. At day 8, the treated groups had significantly ($p \le 0.001$) lower Hb concentration compared with control groups; also the female treated group had a significantly ($p \le 0.001$) lower Hb concentration compared with the male treated group. The Hb value of treated female group was significantly lower compared to the values obtained for the other groups, at weeks 2 and 4 ($p \le 0.001$) and week 3 ($p \le 0.05$). The Hb concentration returned to normal value after 8 days in treated male group and after 3 weeks in treated female group (Fig. 6). There was a significant interaction between T/H and gender for Hb concentration at days 2, 8 ($p \le 0.05$) and week 2 ($p \le 0.01$). (Fig. 6). The interaction indicated that the lowest Hb concentration level was in female group.
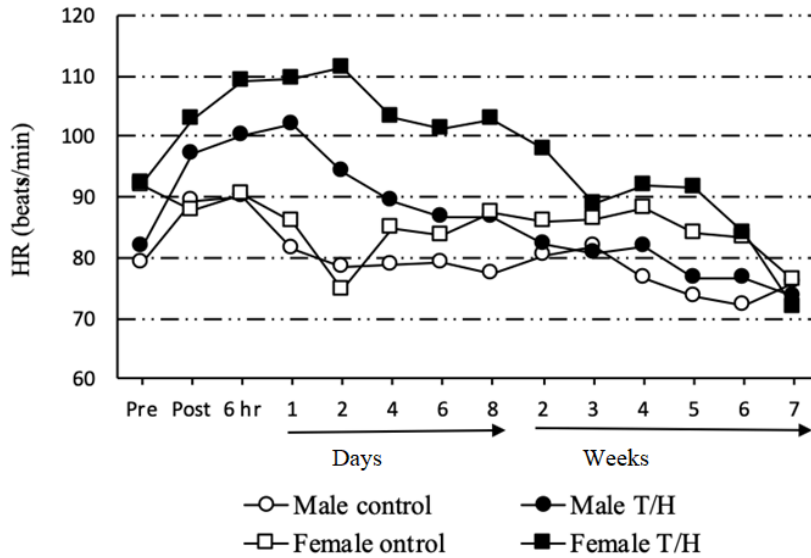

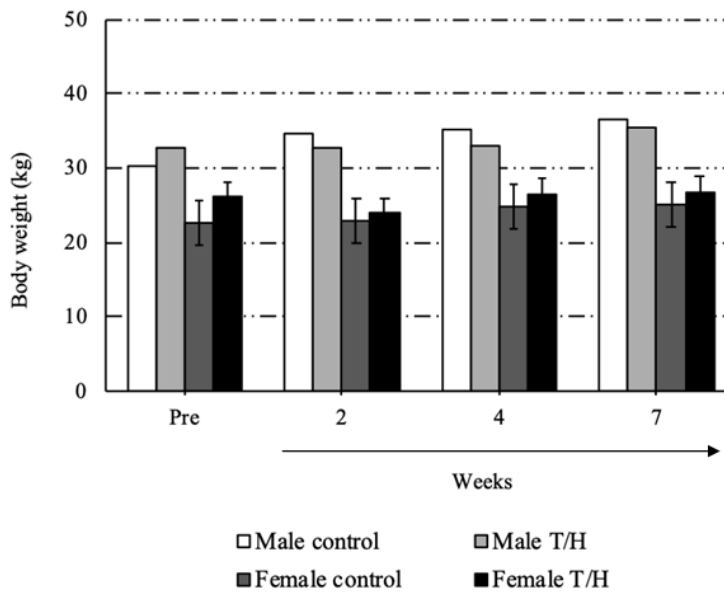Fig.3. Effects of gender and trauma/haemorrhage (T/H) on heart rate, HR in adult goats.

Fig. 4. Effects of gender and trauma/haemorrhage (T/H) on body weight, BW in adult goats.
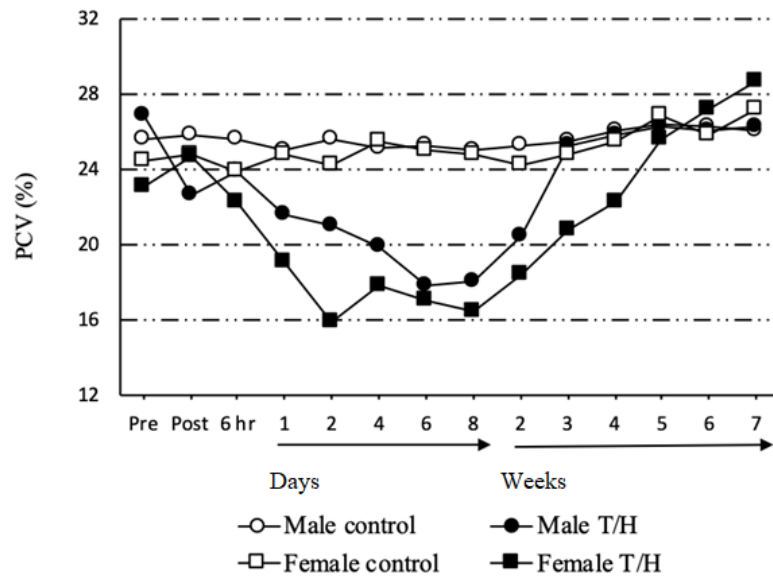
Fig.5. Effects of gender and trauma/haemorrhage (T/H) on packed cell volume, PCV in adult goats.
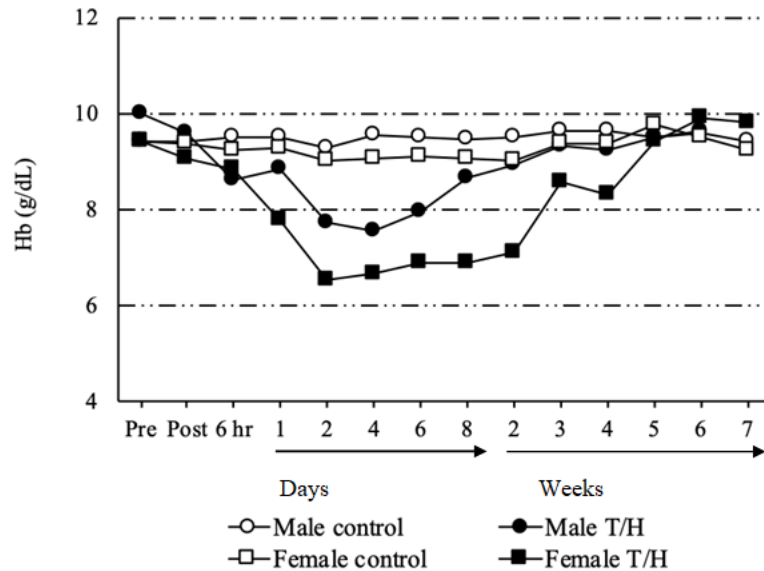

Fig. 6. Effects of gender and trauma/ haemorrhage (T/H) on haemoglobin, Hb concentration in adult goats.

3.7 Total Leukocyte Count (TLC)

At post-T/H, TLC values of both treated groups declined compared with the control groups. The female treated group had significantly ($p \leq 0.001$) lower TLC compared to other groups. At 6 hrs, treated female group had significantly ($p \leq 0.001$) higher and treated male group had significantly ($p \leq 0.001$) lower TLC compared to control groups. The female treated group had significantly ($p \leq 0.001$) higher TLC value compared to other groups, at days 1 and 2. At day 4, both treated groups had significantly ($p \leq 0.001$) higher TLC value compared with the control groups. Also, the treated female group had significantly ($p \leq 0.001$) higher TLC value compared with treated male group. Both treated groups had significantly ($p \leq 0.001$) higher TLC value compared with control groups, at day 6. At day 8, treated groups had higher TLC compared to control groups. The female treated group had significantly ($p \leq 0.01$) higher TLC compared with

control groups. At week 5, treated groups had higher TLC compared to control groups. The TLC values returned to initial values at week 3 in treated male group and at week 4 in treated female group (Fig. 7). There was a significant interaction between gender and T/H on TLC at 6 hrs, days 1, 2 (p≤0.001) and at day 4 (p≤0. 01) with higher TLC in female group.
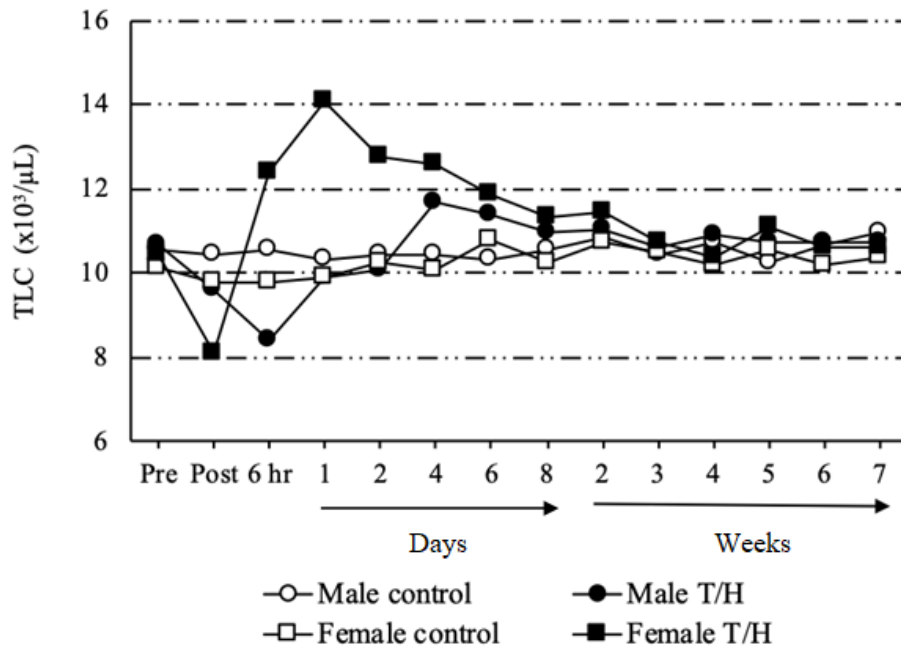

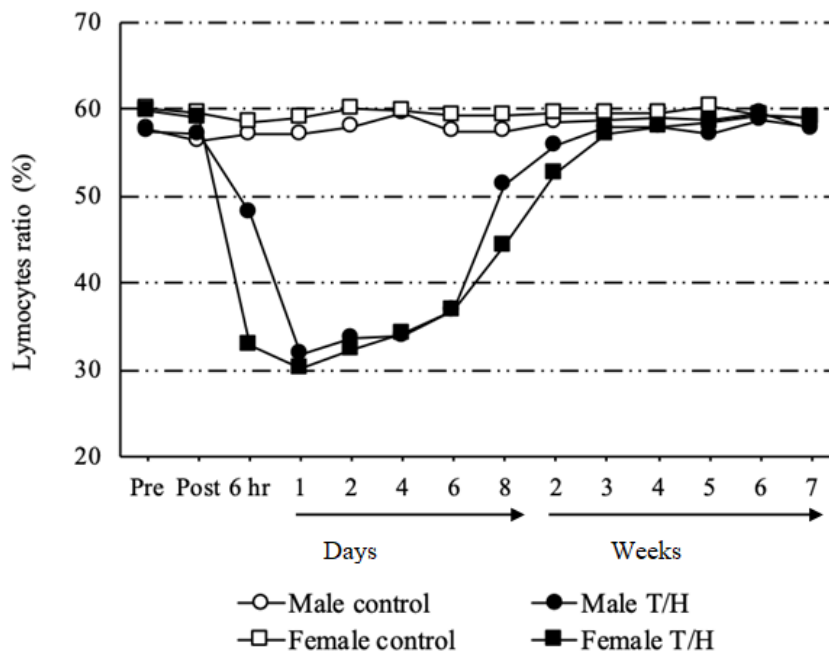Fig.7. Effects of gender and trauma/ haemorrhage (T/H) on total leukocyte count, TLC in adult goats.

Fig.8. Effects of gender and trauma/haemorrhage (T/H) on lymocytes ratio in adult goats.

3.8 Differential Leukocyte Count (DLC)

3.8.1 Lymphocytes ratio

Generally, the treated groups had lower lymphocytes ratio compared with control groups until week 5. At 6 hrs, both treated groups had significantly (p≤0.001) lower lymphocytes ratio compared with the control groups. The female treated group had significantly (p≤0.001) lower lymphocyte ratio compared with male treated group and control groups. The treated groups had significantly (p≤0.001) lower lymphocyte ratio compared with control groups, at days 1, 2, 4 and 6. At day 8, both treated groups had lower lymphocytes ratio, but the female treated group had significantly (p≤0.05) lower lymphocyte ratio compared with the control groups (Fig. 8). There was a significant interaction between gender and T/H at 6 hrs (p≤0.01) indicating lower lymphocyte ratio in female group.

3.8.2 Neutrophil ratio

Both treated groups had significantly (p≤0.001) higher neutrophils ratio compared with the control groups at 6 hrs. The female treated group had significantly (p≤0.001) higher neutrophils ratio compared with male treated group. The treated groups had significantly (p≤0.001) higher neutrophils ratio compared with control groups, at days 1, 2, 4 and 6. At day 2, the female treated group had significantly (p≤0.001) higher neutrophils ratio compared with the male treated group. The female treated group had significantly (p≤0.001) higher neutrophils ratio compared with other groups, at day 8. At week 2, the female treated group had significantly (p≤0.05) higher neutrophils ratio compared with other groups (Fig. 9). There was a significant interaction between gender and T/H on neutrophils ratio at 6 hrs (p≤0.01) and days 1 (p≤0.05) and 8 (p≤0.01) (Fig. 9). The interaction indicated higher neutrophils ratio in female treated group.

3.8.3 Monocytes ratio

The female treated group had lower monocytes ratio compared with other groups at 6 hrs. The treated female group had significantly (p≤0.05) lower monocytes ratio compared with male control group at day 1. At day 2, the female treated group had significantly (p≤0.01) lower monocytes ratio compared with other groups. At day 4, the female treated group had significantly (p≤0.05) lower monocytes ratio compared with the male groups. After day 4, the monocytes ratio showed fluctuations pattern until the end of the experimental period (Fig. 10).
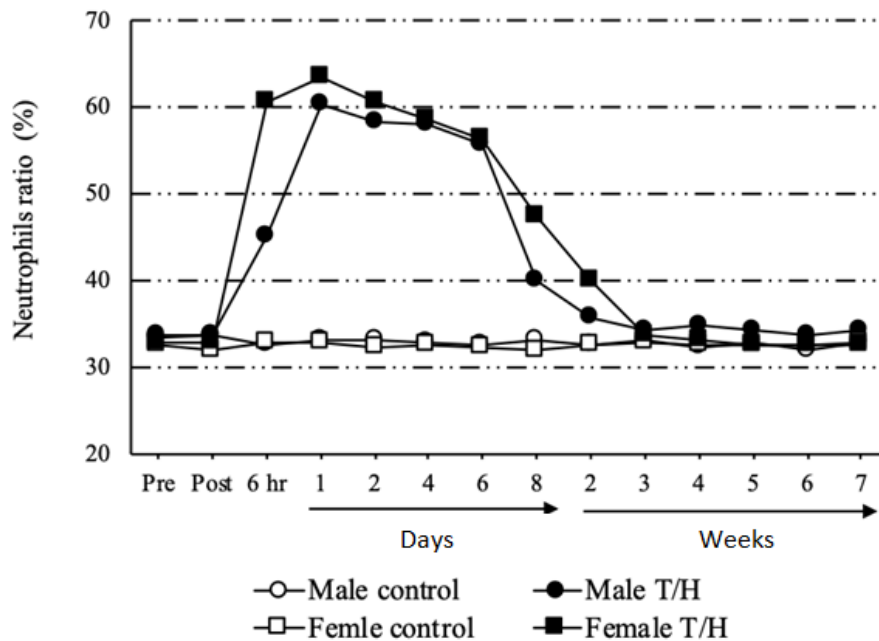

Fig. 9. Effect of gender and trauma /haemorrhage (T/H) on neutrophils rato in adult goats
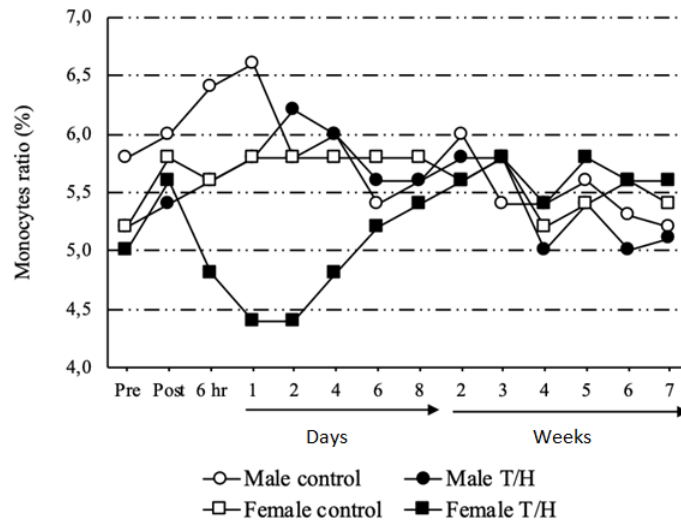
Fig.10. Effects of gender and trauma/haemorrhage (T/H) on monocytes ratio in adult goats.
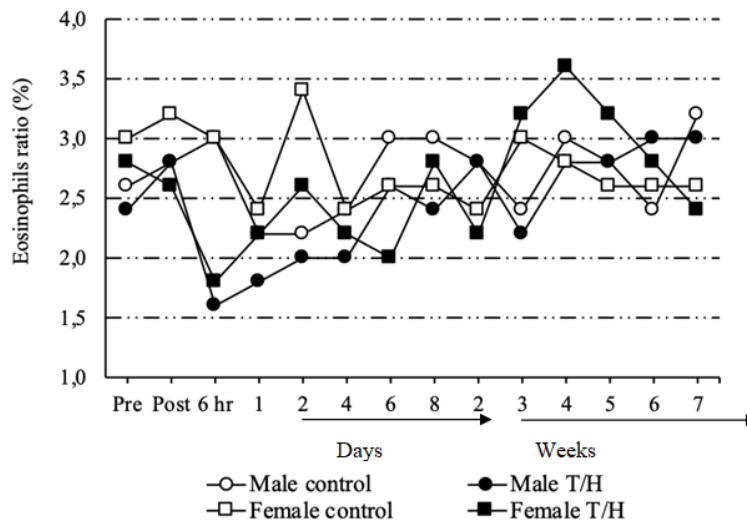

Fig.11. Effects of gender and trauma/haemorrhage (T/H) on eosinophils ratio in adult goats.

3.8.4 Eosinophils ratio

Generally, the eosinophils ratios were lower in treated groups compared with control groups until day 8 in male treated group and week 2 in female group, and. At day 2, both male groups had significantly (p≤0.05) lower eosinophils ratio compared to female control group (Fig. 11).

3.9 Serum Total Protein

At 6 hrs and days 1, 2 and 4, the treated groups had significantly (P≤0.001) lower serum total protein level compared with the control groups. Thereafter, both treated groups maintained lower total protein level compared with respective control groups; female treated group until day 8 and male treated group until week 3 (Fig. 12). There was a significant interaction between gender and T/H on serum total proteins at day 2 (p≤0.05). The interaction indicated lower total protein in female group.

3.10 Serum Albumin

At day 1, both treated groups had significantly (P≤ 0.01) lower albumin concentration compared to control groups. At day 2, the female treated group had significantly (P≤0.05) lower albumin concentration compared to control groups. After day 2, there was no significant difference in

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albumin concentration between all the experimental groups until the end of the experimental period (Fig. 13).

3.11 Serum Urea

The treated groups had significantly ($P \leq 0.001$) higher urea concentration compared with control groups at post-T/H, 6 hrs and day 1. At day 2, both treated groups had significantly ($P \leq 0.05$) higher urea concentration compared with male control group, also the treated groups had significantly ($P \leq 0.05$) higher mean values of urea compared with its respective control. After day 2, there were no significant differences between all groups until the end of experimental period (Fig. 14).
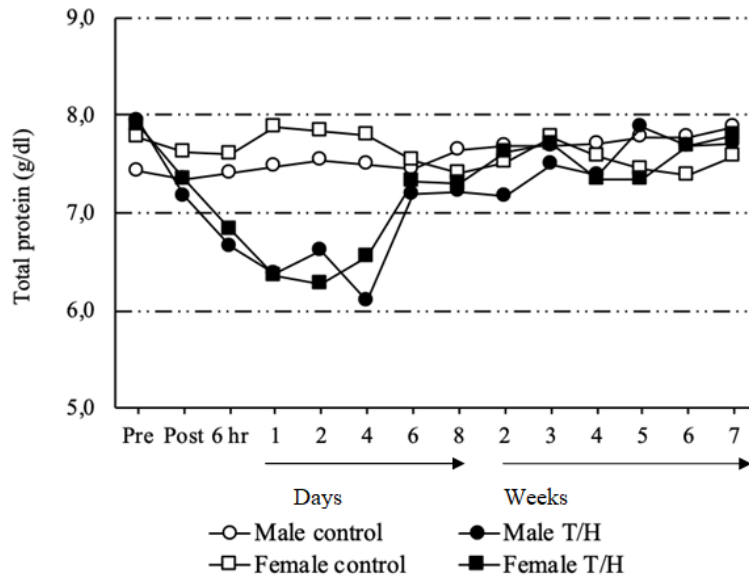

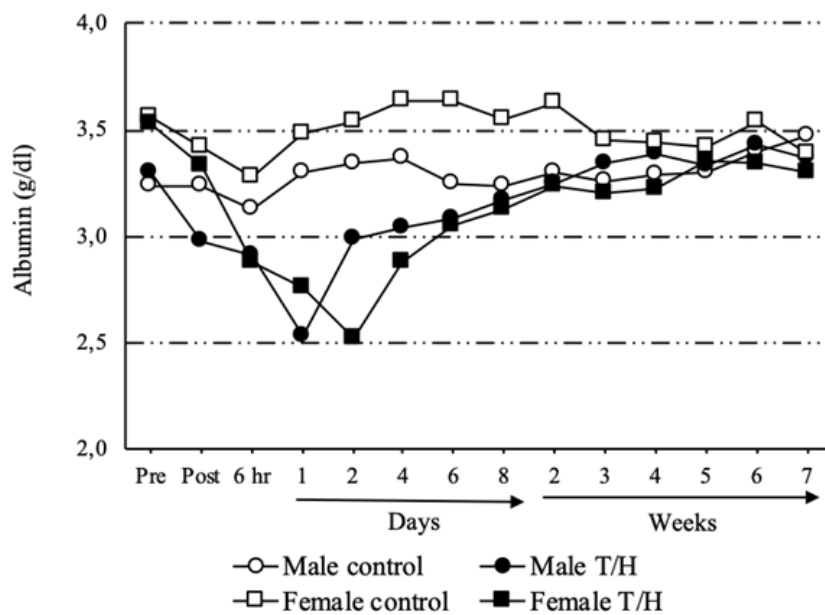Fig.12. Effects of gender and trauma/haemorrhage (T/H) on serum total protein concentration in adult goats.

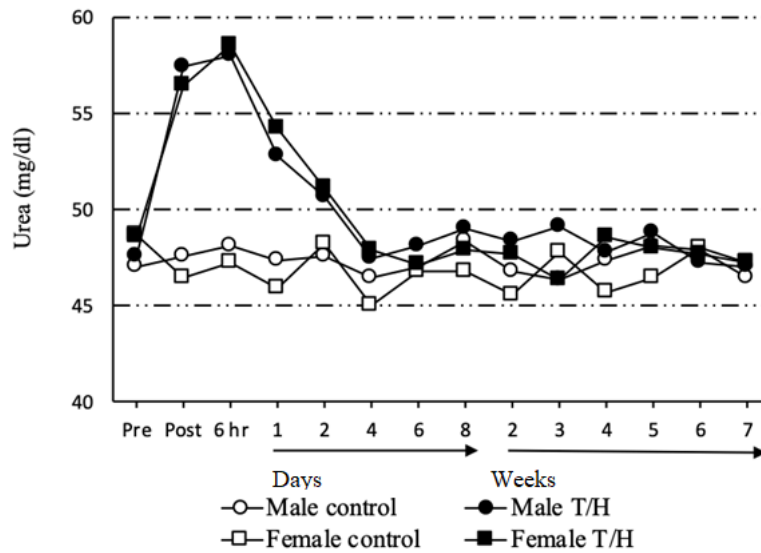Fig.13. Effects of gender and trauma/haemorrhage (T/H) on serum albumin concentration in adult goats.

Fig.14. Effects of gender and trauma/ahemorrhage (T/H) on serum urea concentration in adult goats.
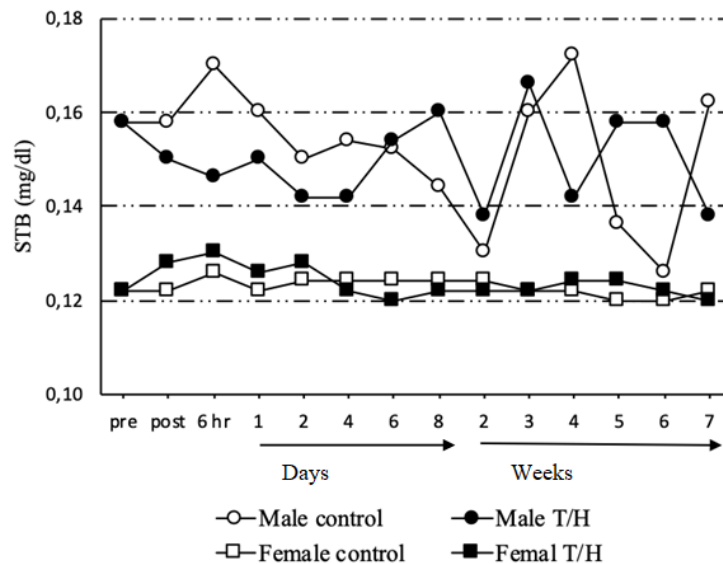
Fig.15. Effects of gender and trauma/haemorrage on serum total bilirubin (STB) concentration in adult goats.

3.12 Serum Total Bilirubin (STB)

At 6 hrs, days 1 and 4, the female groups had significantly (P≤ 0.05) lower STB value compared with the male control group. At day 8, the treated male had higher STB compared to female groups. The male groups had significantly (P≤ 0.01) higher STB values compared with the female groups at day 6. Also, at weeks 2, 3, 4, 5, 6 and 7, the male groups had significantly (P≤ 0.001) higher STB values compared with the female groups (Fig. 15).

3.13 Serum Direct Bilirubin (SDB)

Post-T/H, the treated male group had significantly (P ≤0.01) lower SDB value compared with other groups. At day 2, the male treated group had significantly (P≤0.001) lower SDB concentration compared with female treated group. The male treated group had significantly (P≤0.001) lower serum SDB level compared with female groups at day 4. After day 4, there was no significant difference between groups until the end of the experimental period (Fig. 16). There was significant (p≤0.05) interaction between

gender and T/H in SDB concentration at post-T/H and day 4. The interaction indicated higher SDB in female group.

3.14 Plasma Glucose

Post-T/H and at day 1, treated groups had significantly (P≤0.001) higher glucose level compared with the control groups. Also the treated female group had significantly (P≤0.001) higher glucose concentration compared with male treated group. Both treated groups had significantly (P≤0.001) higher glucose concentration compared with control groups, at 6 hrs and day 2. At day 4, the male treated group had significantly (P≤0.01) higher glucose concentration compared with other groups. At week 3, the male treated group had significantly (P≤0.05) higher plasma glucose concentration compared with female groups (Fig. 17). The interaction between gender and T/H indicated significantly higher glucose concentration at post-T/H (p≤0.01) and day1 (p≤0.001) and a lower value at day 4 (p≤0.05) in female treated group.

Fig.16. Effects of gender and trauma/haemorrhage (T/H) on serum direct bilirubin (SDB) concentration in adult goats.

Fig.17. Effects of gender and trauma/haemorrhage (T/H) on plasma glucose concentration in adult goats.

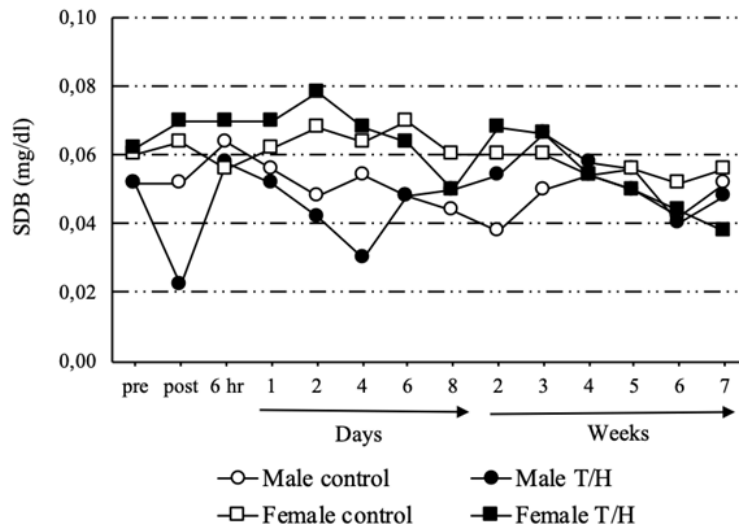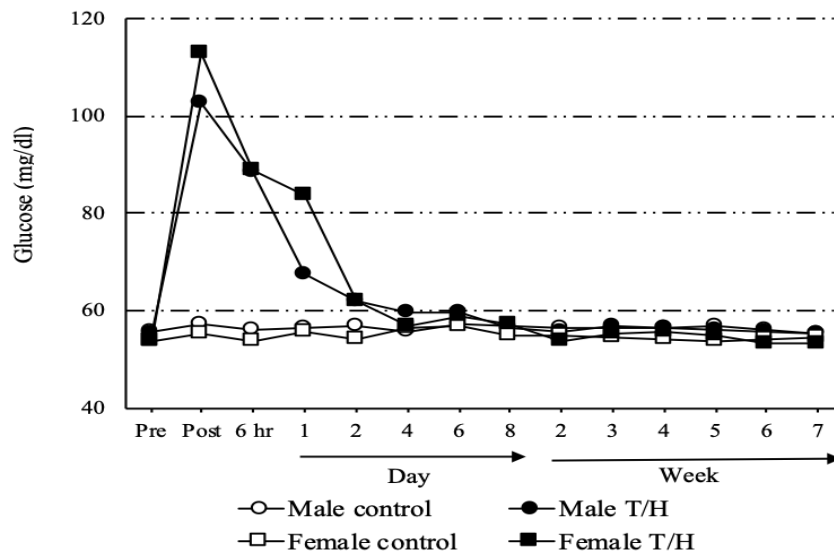# 4. DISCUSSION

The results indicate that the body temperature (T_r) declined in the groups of goats subjected to T/H compared to control groups until 6 hrs and day 2 in female and male treated groups, respectively (Fig. 1). Although T_r remained within the normal range of the species (38- 40⁰C) [33], the decrease in T_r post-T/H is probably due to the effects of both trauma and haemorrhage. Hypothermia is usually associated with haemorrhage which reduces availability of oxygen and substrates, and stimulates anaerobic metabolism leading to decrease in ATP synthesis and subsequent heat production [34]. Also, the physiological responses to trauma involve insulin resistance combined with the decrease in insulin secretion; this leads to decreased glucose uptake by cells [35]. At the cellular level, ATP depletion plays a major role in the pathophysiology of spontaneous hypothermia [36]. Brown et al. [37] reported that the thermoregulatory set point decreased due to a drop in body core temperature after haemorrhage in rats. In contrast .Abdalla and Abdelatif [38] reported an increase in T_r in adult goats subjected to 30% haemorrhage that was related to a decrease in convective heat transfer to the periphery and peripheral vasoconstriction associated with the secretion of catecholamines.

Following T/H, T_r decreased significantly in the female treated group. The male treated goats had significantly higher T_r compared to the female treated group; this is presumably due to the effect of androgens on heat production and energy expenditure. Woods et al. [39] reported that testosterone improves oxygen uptake throughout the body. In intact goats, males had higher body temperatures compared with females [40]. Oestrogens inhibit cold and stimulate warm-sensitive neurons, leading to inhibition of the heat-retaining mechanism and exciting heat loss mechanism, thus causing a decrease in the regulated body temperature [41]. Previous studies [42,43] reported that oestrogens generally promote vasodilatation in the skin, resulting in greater heat dissipation and lower body temperature in women.

The current results indicate that T/H increased the respiratory rate (RR) until 6 hrs and day 1 in female and male goats, respectively (Fig. 2). This finding may be attributed to a lack of adequate oxygenation and an increase in the production of lactic acid which stimulates the respiratory centre [44]. Argolo et al. [45]

attributed hyperventilation in goats subjected to 30% blood volume loss to the sensitivity of the respiratory centre to the concentration of hydrogen ions produced when there is a reduction in oxygen transport. Fournier et al. [46] reported that hypoxia increased the breathing frequency in rats.

The higher RR in the female treated group compared with the male treated group following T/H and at 6 hrs may be attributed to the effects of female sex hormones on the respiratory system. Previous studies reported that ovarian hormones are potent stimulants of ventilation, especially progesterone in humans [47] and rats [48]. Golparvar et al. [49] showed that the administration of progesterone increases ventilatory performance in adult human trauma patients.

The present results indicate that T/H increased the HR until week 2 in males and week 6 in female goats (Fig. 3). This is likely due to activation of the sympathetic nervous system that resulted in increased secretion of catecholamines from the adrenal medulla and release of norepinephrine from presynaptic nerve terminals [44]. The increased sympathetic activity results in tachycardia and hypertension in trauma [50]. The decline in blood volume produced by bleeding decreases venous return, cardiac output and increases heart rate [51]. Acute loss of 30% of blood volume increased the HR in goats [45].

The male treated goats had significantly lower HR mean values compared with the female treated group. The difference between male and female heart function may be attributed to the effects of sex steroids. Female sex hormone, oestradiol has salutary effects on depressed organ functions after T/H and resuscitation [4, 23]. 17β-oestradiol improved cardiac function following T/H in male rats [52]. Also, Hsieh et al. [12] reported that cardiac function of adult male rats was depressed markedly after trauma and haemorrhage. The current results in Nubian goats are in agreement with previous findings [21, 53] who reported that cardiac index was depressed in males compared with females in pro-oestrus state following T/H and resuscitation in rats.

The body weight (BW) decreased in both male and female goats subjected to T/H until week 2 compared to respective control groups (Fig. 4). This response is presumably attributed to stress

following T/H and increased secretion of catabolic hormones that result in substrate mobilization and loss of muscle protein [54]. Glucocorticoid- induced muscle atrophy results from increased protein breakdown and decreased protein synthesis [55]. A concomitant decrease in feed intake in response to stress might have also contributed to the observed weight loss of treated goats.

The present results indicate that T/H decreased the PCV in treated goats compared to control groups (Fig. 5). This decrease is attributed to blood loss and decrease in blood volume which lead to movement of fluids from interstitial space to blood stream to restore blood volume [38]. Also, the decrease in PCV may be related to post- trauma responses which increase antidiuretic hormone and aldosterone secretion with consequent retention of water and salt that causes expansion of extracellular fluid leading to haemodilution [56]. A significant decrease in PCV was observed after one week of surgery in goats subjected to laparotomy [24].

The obtained results indicate that the PCV was lower in female treated goats compared to male treated goats. This is probably due to the effects of sex hormones. In intact goats, males had higher PCV compared with females [57]. Androgens could stimulate erythropoiesis and increase the values of erythrocytes, Hb and PCV [58, 59]. Previous studies [21 ,60] reported lower PCV value after T/H in male rats.

The findings of the present study indicate that the Hb concentration decreased in both male and female goats subjected to T/H (Fig. 6). This decrease could be due to blood loss anaemia associated with hypovolemia and reduction in oxygen-carrying capacity of blood [61]. Haemorrhage in trauma patients was associated with an early decrease in Hb level [62]. The lower Hb concentration in female treated goats compared with male treated group is presumably related to the effect of sex hormones. Usually intact males have higher Hb concentration compared to intact females [63].

The TLC decreased in both treated goats compared with respective control values post-T/H (Fig. 7). This response may be attributed to trauma stress and increased cortisol secretion. Postoperative immune suppression is characterized by significant decrease in leukocytes count and increased corticosterone levels in mice [64]. The reported decrease in TLC

may also be attributed to haemodilution, which is a physiological response to blood loss [38]. Also, the current findings indicate that the TLC increased in female treated group from 6 hrs until week 3, while the TLC increased in male treated group from day 4 to week 6 (Fig. 7). This could be related to the effects of sex steroids hormones on responses to T/H. Studies indicate that cell-mediated immunity functions are markedly depressed in males but not in proestrus females after T/H [65]. In humans, female patients have higher TLC than male patients at days 1and 3 post-operative [66].

The present results indicate that in treated groups, the lymphocytes, monocytes and eosinophils ratios decreased associated with an increase in neutrophils ratio in response to operation (T/H) compared with the values of control groups. (Figs. 8, 9, 10 and 11). The stress of T/H increases the secretion of glucocorticoid hormones [67]. Elevated cortisol increases the number of neutrophils and decreases the number of circulating eosinophils by increasing their sequestration in the spleen and lungs in humans. Furthermore, glucocorticoids decrease the circulating lymphocyte count and the size of the lymph nodes and thymus by inhibiting lymphocyte mitotic activity [51]. Brochnrt and Toft [68] reported that IL-6 activates neutrophils and inhibits the apoptosis of neutrophils following trauma.

The female treated groups had higher neutrophils and lower lymphocytes ratio compared to male treated group. [69] indicated that 17β-oestradiol prevents neutrophils infiltration and organ damage following T/H in mice. It was also found that 17β-oestradiol administration after T/H reduces tissue neutrophil sequestration in male rodents [70]. Following T/H, T-cells proliferation and function decreased and apoptosis increased [14]. In humans, female patients had more circulating neutrophils, less lymphocytes and monocytes than male patients post-operative [66].

Previous studies have demonstrated a gender specific immune response after T/H [4]. In contrast to male mice, macrophage and lymphocyte responses are maintained or enhanced after T/H in female animals in the proestrus state of the cycle [71]. In mice models , the ratio of lymphocytes decreased and both monocytes and neutrophils were not influenced by surgical trauma [64]. In contrast, in trauma-

hypovolaemic human patients, the TLC, neutrophils and band neutrophil cells remained higher [72].

The current results indicated that the serum concentrations of total protein and albumin decreased significantly in treated goats as from 6 hrs until day 4 (Figs. 12 and 13). This response is mainly related to haemodulation and tissue catabolism. Also it could be attributed to activation of water retention mechanisms. Wintour et al. [73] reported that plasma vasopressin concentration increases after haemorrhage in sheep. Protein catabolism is stimulated by increased cortisol concentrations following T/H. The amino acids may be further catabolized for energy or used in the liver to form new protein, particularly acute phase proteins [50]. The liver also converts amino acids into other substrates, glucose, fatty acids or ketone bodies [67]. Serum albumin concentration, a marker of liver function, was significantly reduced after T/H. Hubner et al. [74] indicated that albumin values drop immediately after surgery. Oestradiol administration after T/H prevents the decrease in serum albumin concentration in male rats [60]. However, studies in rats [75] revealed that bleeding was associated with total protein loss and decrease in albumin and all globulin fractions concentrations.

The serum urea concentration increased significantly in treated groups immediately post-T/H until day 2. The female treated group had higher urea concentration compared with male treated group (Fig. 14). This increase in urea concentration following T/H is attributed to increase in the secretion of catabolic hormones. Increased cortisol concentration following T/H stimulates breakdown of proteins in skeletal and visceral muscles [50]. Protein breakdown significantly increases after surgery in humans [76]. The increase in urea concentration might also be associated with drop in hydrostatic pressure due to hypovolaemia which causes filtration fraction to diminish. The activation of renin-angiotensin-aldosterone system causes efferent arteriole constriction, resulting in a decrease in glomerular filtration rate (GFR), decreased urine production and urea elimination [21, 35]. The observed higher urea concentration in the female treated group could be associated with loss in BW following T/H.

The total bilirubin and direct bilirubin concentration increased in female treated group and decreased in male treated group following T/H (Figs. 15 and 16). These changes may be attributed to the variable effects of sex steroid hormones. Fevery [77] reported that gender influences bilirubin concentration. The conjugated (direct) bilirubin is higher and unconjugated (indirect) bilirubin is lower in female than male goats [78]. This difference might be due to the effects of oestrogen, progesterone and of testosterone on the conjugation rate, because testosterone down-regulates conjugation enzyme, whereas the combination of oestrogen-progesterone enhances enzyme activity [79]. In the serum of normal individuals, the concentration of indirect bilirubin is lower in females than in males [80, 81]. Studies on male mice [82] showed that total bilirubin concentration increases following bone fracture, tissue trauma, and haemorrhage.

The plasma glucose concentration increased significantly post-T/H in both male and female goats compared to the control groups. Until day 2, the female treated group had higher glucose concentration compared with male treated group (Fig. 17). The cortisol released in response to trauma leads to a delay in the metabolism and utilization of glucose, while the increased plasma epinephrine causes gluconeogenesis in liver and skeletal muscle, mobilization of free fatty acids, and insulin resistance, preventing the uptake of glucose [83, 84]. These processes result in a marked increase in plasma glucose concentration. Hyperglycaemia after T/H is facilitated by catecholamines and cortisol hormones, which increase hepatic glycogenolysis and gluconeogenesis [85]. Burton et al. [67] reported that hyperglycaemia may be due to decrease of insulin secretion by the sympathetic nervous system which inhibits pancreatic β-cell secretion. Insulin resistance of peripheral tissues also occurs, reducing the utilization of the available glucose and enhancing the hyperglycaemia [86]. The current finding in goats is in line with Bahten et al. [72] who reported that hyperglycaemia develops in most human patients of T/H.

Experimental data from *in vitro* studies suggest that insulin and sex hormones interact. Sex steroids and insulin interact in various parts of physiological field. Previous studies [87] indicated that there is a positive correlation between serum testosterone concentration and insulin sensitivity in humans. Oestrogens influence insulin resistance, insulin sensitivity and ß-cell function in rats [88,89]. Progesterone also plays significant roles in insulin physiology in

rats [90]. Almadi and Orya [91] suggested that in rats, oestradiol is serum insulin enhancer hormone and progesterone is serum insulin reducer hormone.

5. CONCLUSION

The goat model was adopted successfully in studying the pathophysiological consequences of trauma / haemorrhage (T/H) in relation to gender (sex). For each sex, there were marked changes in thermoregulation, haematological parameters and blood biochemical constituents investigated. Gender influenced the physiological responses of goats to T/H illustrated by significant leukocytosis, neutrophilia, lymphopenia and marked hperglycaemia in females compared to males.

CONSENT

It is not applicable.

ETHICAL APPROVAL

This study was approved by the Research Board, Faculty of Veterinary Medicine, University of Khartoum and approved and monitored by the Ethical Committee of Veterinary Council, Sudan.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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