Effects of Immobilization , Heat Stress and Antioxidant Supplementation on Thermoregulation and Haematological Responses in Male Rabbits (*Oryctolagus cuniculus*)

ABSTRACT

Background and Objectives : Immobilization stress may induce negative effects on physical and physiological activities of humans and animals. Heat stress also influences the health and survival of mammalian species , particularly under tropical conditions . The objective of the study was evaluation of the responses to immobilization (IMO) and acute heat stress (HS) in a rabbit model . The potential protective effects of administration of antioxidants on IMO and acute heat stress (HS) were also assessed . **Materials and Methods** : Sixty six male rabbits (mean body weight , BW :1582±28g) were used in three trials to investigate the effects of HS , IMO+HS and administration of vitamin C (IMO+HS +Vit C) or vitamin E-selenium (IMO+HS+VitaminE-Se). Immobilization was performed by fixing the animals in a specially designed box ; HS was induced by exposing rabbits to direct solar radiation (370 W/m2) for 1 hour (trial 1) and 2 hours (trials 2 and 3). The body weight (BW) , rectal temperature (Tr) and heart rate (HR) were monitored and venous blood samples were collected before the beginning of the trial and then at 2, 24 and 48 hours after the end of the trial. The packed cell volume (PCV) , total leukocytes count (TLC) and differential leukocytes count

(DLC) were determined . **Results** :In trial- I, 18 rabbits were randomly assigned to 3 groups of 6 each (control, HS and IMO+HS). HS rabbits showed higher values of HR (P<0.01) compared to IMO+HS rabbits . In trial -II , 24 rabbits were assigned to 4 groups comprising control, HS, IMO+HS (received 2 doses of normal saline) and IMO+HS +Vit. C (received 2 doses of 300 mg/kg/BW each Vit.C subcutaneously). IMO+HS animals had higher responses compared to HS, as evidenced by significantly (P<0.01) higher values of Tr and HR. Administration of Vit. C decreased Tr, and maintained HR and haematological parameters relatively constant. In trial 3, 24 rabbits were assigned to 4 groups comprising control(received 2 doses of normal saline s/c) , HS, IMO+HS and IMO+HS +Vit. E-Se (received 2 doses 100 mg/kg/BW each Vit E-Se s/c). The responses of animals to IMO+HS were greater compared to HS alone. IMO+HS significantly (P<0.001) increased Tr and HR. Furthermore, IMO+HS rabbits showed significant (P<0.001) decreases in PCV and TLC after 48hrs and 24 hours, repectively, compared to the values of control rabbits. Administration of Vit.E-Se decreased Tr, HR and maintained haematological parameters relatively constant. **Conclusion** : Immobilization aggravated the negative effects of heat stress, while Vit. C was more effective than Vit.E-Se in alleviation of hyperthermia and maintaining normal haematological parameters in rabbits .

Keywords: Rabbit ; Immobilization ; Heat stress ; Antioxidants ; Thermoregulation ; Blood constituents .

1. INTRODUCTION

Stress is associated with increased incidence of morbidity and mortality rates in animals and humans. The induced oxidative stress influences body homeostasis [1] which plays a major role in prevalence of several health problems that include cardiovascular diseases [2] , hypertension, and other metabolic disorders [3]. Exposure of rabbits to high environmental temperature caused disturbances in blood parameters, enzymatic reactions and hormonal secretions [4-6] . Under certain circumstances HS could be associated with IMO stress. IMO has been considered as an acceptable protocol for physical and psychological stress in mammals [7,8] . It could be associated with several physiological and haematological changes involving leukocyte and erythrocytes [9 -11].

Micronutrients and antioxidant substances, primarily Vitamin C , Vitamin E and selenium(Se) were used to alleviate various forms of stress including IMO [12] , restraint [13 ,14] and HS [15]. Immobilization in humans and animals for a prolonged time as in cases of physical disability is associated with several physiological disorders related to responses of hypothalamic–pituitary–adrenal (HPA) axis. There is paucity of information regarding the combined effect of heat and immobilization stress and alleviation by supplementation of antioxidants. Accordingly,this study aimed to adopt the rabbit model to evaluate the responses to immobilization and heat stress and potential beneficial effects of administration of Vitamin C or Vitamin E+Se .

2. MATERIALS AND METHODS

2.1 Animals, Housing, Feeding and Management

Sixty six (66) mature male rabbits with an average BW of 1582+28g were used . Animals were kept in the animal house at the Department of Physiology, Faculty of Veterinary Medicine , University of Khartoum in individual cages and were allowed to adapt to the experimental procedures for two weeks. During the adaptation period, animals were given access to food and tap water *ad libitium*. Animals were given fresh lucerne (*Medicago sativa*) and a rich source of starch (Sorghum grains). All animals were given a prophylactic dose of anthelmintic injection (Ivermectin 0.02 ml/kg BW) and antibacterial injection (Oxytetracycline: 7.5 mg/kg BW).

2.2 Immobilization of animals

Immobilizations stress was induced using a specially designed wood box (102 x 32 x 22 cm). The box was divided into 6 individual chambers and supplied with horizontal tape to restrain the animals .During experimental periods , animals were placed inside the immobilization device and fixed gently, with their heads outside the chambers .

2.3 Rectal temperature (Tr) , heart rate (HR) and body weight (BW)

The ambient temperature (Ta), relative humidity (RH) and wind speed (WS) measurements were obtained from the nearest Meteorological station . The Tr was measured using a digital thermometer, while the HR of animals was monitored using a stethoscope and stopwatch.

2.4 Haematological Parameters

Standard haematological methods described by Jain [16] were used for measuring the haematological parameters , PCV, Hb concentration , total leukocyte count (TLC) and differential leukocyte count (DLC) .

2.5 Experimental Design

In trial-I , 18 rabbits were assigned to three groups with equal numbers : control group rabbits were on free movement under shade, HS rabbits were on free movement and subjected to heat stress by exposure to direct solar radiation for 1hour, HS+IMO rabbits were subjected to the specified treatments for 1hour. In trial-II , 24 rabbits were randomly assigned to 4 groups with equal numbers : control rabbits were on free movement under shade, HS rabbits were on free movement and subjected to heat stress for 2hrs, HS+IMO animals were injected with normal saline and then subjected to HS+IMO stress for 2 hrs, and HS+IMO+Vit.C treated, HS+IMO+Vit.C rabbits received 2 doses of 300mg(s/c) of Vit. C/kg (Troy Laboratories PTY, Ltd , Australia) .The first dose was injected one week prior to the experiment and the second dose was injected immediately before the animals were subjected to HS +IMO for 2 hrs. The initial baseline values for thermoregulation were obtained and blood samples were taken before the beginning of the trial and then at 2, 24 and 48 hours after the end of the treatments . In trial-III , 24 rabbits were randomly assigned to four groups with equal numbers : control rabbits were on free movement under shade, heat stressed (HS) rabbits were on free movement and subjected to heat stress for 2hrs, heat stressed +immobilized (HS+IMO) rabbits were injected with normal saline and then subjected to HS+IMO stress for 2hours , and HS , IMO and Vit.E+Se (IMO+HS+Vit.E-Se) rabbits were pre-administered two doses of 100mg/kg Vit.E–Se (Fravet Laboratories B.V., Netherlands) each s/c . The first dose was injected one week before the treatment while the second dose was injected immediately before subjecting animals to HS and IMO for 2 hours. For all trials**,** the initial baseline values for thermoregulation were obtained and blood samples were taken before the onset of the experiment and then at 2, 24 and 48 hours after the end of exposure to treatments.

2.6 Statistical Analysis

The data were analysed using statistical analysis SAS – 2002 software [17] . The analysis of variance (ANOVA) and Duncans Multiple Range Test (DMRT) were used to evaluate the effects of HS , HS+IMO and supplementation of antioxidants on the parameters investigated . The difference between mean values was separated by least significant difference (LSD) test. The results are presented as mean±SD and the $P<0.05$ was considered statistically significant.

3. RESULTS

3.1 Effects of Acute HS and IMO for One Hour

3.1.1 Climatic conditions

The data of Ta , RH and WS during the experimental period (November and December, 2015) are presented inTable1.

3.1.2 Rectal Temperature and Heart Rate

The effects of HS and IMO+HS on Tr and HR are presented in Table 2. There was a significant ($P<0.001$) increase in Tr in HS and IMO+HS rabbits compared to the control group value. The mean value of Tr for IMO+HS rabbits was higher than that for HS rabbits.The HR was significantly increased in HS ($P<0.01$) and IMO+HS ($P<0.05$) rabbits compared to the control rabbits.

3.1.3 Packed Cell Volume and Total Leukocyte Count

Table 3 shows the effects of HS and IMO+HS on PCV and TLC .There was no significant difference in PCV of HS and IMO+HS rabbits during the experimental period. However, the data showed a slight decrease in PCV of HS rabbits and a slight increase in PCV of IMO+HS rabbits compared to the respective control values. The TLC was non-significantly decreased in HS and IMO+HS rabbits compared to the control group rabbits. The decrease was more pronounced in IMO+HS rabbits than in the HS rabbits.

3.1.4 Differential Leukocyte Count

The effects of HS and IMO+HS on DLC are illustrated in Table 4. The data indicate non-significant difference in the percentage of lymphyocytes and neutrophils of HS and IMO+HS rabbits compared to respective control group values.The monocyte percentage was non-significantly different between HS and IMO+HS rabbits compared to the mean value of the control group rabbits. However, the data showed that in IMO+HS rabbits, the monocyte percentage was slightly decreased compared to the control rabbits. The eosinophil percentage of IMO+HS rabbits was slightly decreased after the treatments compared to the value of the control group rabbits. The basophil percentage was slightly increased in IMO+HS rabbits compared to the respective values of the control rabbits.

3.2 Effects of HS , IMO and Administration of Vitamin C.

3.2.1 Rectal temperature and heart rate

The results of the effect of HS, IMO+HS and IMO+HS and administration of Vit. C on Tr and HR are presented in Table 5. Tr was significantly ($P<0.001$) increased in HS, IMO+HS and IMO+HS+Vit. C rabbits after 2hrs, and in IMO+HS rabbits ($P<0.01$) after 24 and 48 hrs compared to the respective mean value of control rabbits. Tr values were highest in IMO+HS rabbits throughout the experimental period. Vit. C administration normalized Tr of IMO+HS + Vit. C treated rabbits . The HR was significantly increased in HS rabbits ($P<0.01$) after 24hrs, and in IMO+HS rabbits after 2hrs ($P<0.01$) and 24hrs ($P<0.001$) compared to the respective control rabbits. The HR was highest in IMO+HS rabbits throughout the experiment. Administration of Vit. C maintained the HR of IMO+ HS+Vit. C treated rabbits.

3.2.2 Packed Cell Volume and Total Leukocyte count

The effects of HS, IMO+HS and IMO+HS + Vit. C on PCV and TLC are presented in Table 6. The PCV was significantly ($P<0.05$) lower in IMO+HS rabbits after 48hrs compared to the respective control value . The pattern indicates that the PCV of IMO+HS + Vit. C treated rabbits was slightly higher after 2hrs, and then slightly lower after 24hrs compared to the control group at the same time points. The TLC was significantly ($P<0.01$) decreased in IMO+HS rabbits after 2hrs, significantly increased in HS rabbits after 24hrs ($P<0.01$) and 48hrs ($P<0.05$), and in IMO+HS + Vit. C treated rabbits after 24hrs ($P<0.05$) compared to the control group values . The TLC was lowest in IMO+HS rabbits throughout the experimental period, and Vit. C administration relatively maintained the TLC in rabbits.

3.2.3 Differential Leukocyte Count

The effects of HS, IMO+HS and IMO+HS + Vit. C on DLC in rabbits are presented in Table 7. The lymphocyte percentage was significantly increased in HS rabbits after 24hrs ($P<0.01$) and 48hr ($P<0.05$). In IMO+HS rabbits, a significant ($P<0.05$) decrease was obtained after 24hrs , however , a significant ($P<0.05$) increase was obtained in the same experimental group after 48hrs. Also there was a significant ($P<0.05$) increase in lymphyocyte percentage of IMO+HS + Vit. C rabbits after 48hrs compared to the respective control values. There was a significant decrease in neutrophil percentage in HS rabbits after 24hrs ($P<0.01$) and 48hrs ($P<0.05$). In IMO+HS rabbits, the value was significantly ($P<0.05$) increased after 24hrs, however, it was significantly ($P<0.01$) decreased after 48hrs. In IMO+HS + Vit. C rabbit, a significant ($P<0.01$) decrease was obtained after 24 and 48 hrs compared to the respective control values. The monocyte percentage was slightly decreased in HS rabbits after 2hrs compared to the respective mean value of control rabbits. The results indicate that the eosinophil percentage was significantly ($P<0.05$) decreased in

IMO+HS rabbits after 48hrs compared to the control rabbits. The basophil percentage decreased significantly ($P<0.05$) in HS rabbits after 2 hrs compared to the respective control group value.

3.3 Effect of HS, IMO and Administration of Vit. E-Se

3.3.1 Rectal Temperature and Heart Rate

Table 8 shows the effects of HS, IMO+HS and IMO+HS+Vit. E-Se on Tr and HR in male rabbits. Tr was significantly increased in HS rabbits after 2hrs ($P<0.001$), in IMO+HS rabbits after 2hrs ($P<0.001$) and 48hrs ($P<0.05$), and in IMO+HS + Vit. E-Se rabbits only after 2hrs ($P<0.01$) compared to the respective control group values . Administration of Vit. E-Se maintained Tr of IMO+HS +VitE-Se after 24 and 48 hrs. The data indicate that the HR was significantly ($P<0.01$) increased in IMO+HS rabbits after 2 hrs , 24 hrs and 48 hrs compared to the respective control group values. In HS rabbits, there was a slight increase in HR after 2hrs and 24 hrs. A non-significant increase was also obtained in IMO+HS + vitamin E - Se rabbits after 2 hrs and 24 hrs. Administration of vitamin E - Se maintained the HR of IMO+HS +Vit.E-Se rabbits relatively constant.

3.3.2 Packed Cell Volume and Total Leukocyte Count

The effects of HS, IMO+HS and IMO+HS+Vit. E-Se on PCV and TLC in male rabbits are presented in Table 9. The pattern indicates that the PCV of HS and IMO+HS rabbits was slightly decreased after 2 and 24 hrs and the PCV of IMO+HS+Vit. E-Se rabbits was slightly increased after 2hrs compared to the respective control group values. The PCV of IMO+HS rabbits maintained the lowest value throughout the experimental period.The TLC decreased significantly ($P<0.01$) after 2hrs and then increased after 24hrs in HS rabbits. A non-significant decrease was obtained in IMO+HS

rabbits after 2hrs and a significant (P<0.01) decrease was reported after 48hrs. In IMO+HS+Vit. E-Se rabbits, TLC was decreased after 2hrs, and the values remained lower after 24 hrs and 48 hrs compared to the respective control group values. In IMO+HS +Vit. E-Se rabbits,TLC values were relatively maintained compared to the other experimental groups .

3.3.3 Differential Leukocyte Count

Table 10 shows the effect of HS, IMO+HS and IMO+HS+Vit. E-Se on DLC . The lymphocyte percentage decreased significantly in HS rabbits after 2hrs (P<0.05), and in IMO+HS rabbits after 2hrs (P<0.05) and 24hrs(P<0.01). The lymphocyte percentage was lowest in IMO+HS rabbits throughout most of the experimental period. Administration of Vit. E-Se alleviated the lymphopenia induced by IMO+HS. There was a significant (P<0.01) increase in neutrophil percentage of HS rabbits after 2hrs. The data also indicate a significant (P<0.01) increase in neutrophil percentage of IMO+HS rabbits after 2hrs compared to the respective control group values . Administration of Vit. E-Se ameliorated the neutrophilia induced by IMO+HS.The monocyte percentage was significantly (P<0.01) decreased in HS rabbits and non-significantly decreased in IMO+HS rabbits after 2hrs.Administration of Vit. E-Se maintained the monocyte percentage induced by IMO+HS. The eosinophil percentage was significantly (P<0.05) decreased in HS rabbits after 2hrs, followed by non-significant increase after 24 hrs and 48 hrs. In IMO+HS rabbits, the eosinophil percentage was significantly increased (P<0.05) after 24hrs compared to the control value. The pattern indicates that the eosinophil percentage of IMO+HS rabbits decreased after 2hrs, and increased after 48hrs. The eosinophil percentage increased non-significantly in IMO+HS+Vit. E-Se rabbits after 48hrs. However, administration of Vit. E-Se maintained the eosinophil value relatively constant after 2hrs and 24hrs. The basophil

percentage decreased in IMO+HS rabbits after 24 hrs. Administration of Vit. E-Se slightly reversed the change in basophil value induced by IMO+HS.

4.DISCUSSION

4.1 Thermoregulation and Heart Rate :

The results showed marked hyperthermia in all groups of rabbits exposed to HS (Tables 5, 6 and 8). Hyperthermia was more remarkable in IMO+HS rabbits , however, IMO+HS+Vit.C and IMO+HS+Vit.E+Se rabbits exhibited a slight increase in Tr. Increased thermal load enhanced heat gain from the surrounding leading to heat stress [18] . Thermoregulation in rabbits was directly influenced by thermal environments [19] .The sensible heat loss becomes non-effective at high ambient temperature and is replaced by evaporative heat loss through panting . Furthermore, heat generated by the respiratory muscles activity during panting may contribute to the high core temperature [20,21] . The reduction in Tr associated with micronutrient supplementation (Tables 6 and 9) is presumably attributed to the antioxidant effects of both Vit. C and Vitamin E in protecting the biological membranes against the lipid peroxidation by reactive oxygen species , ROS [22]. An increase in Tr of rabbits submitted to heat stress, decreased significantly on administration of Vitamin E – Se [15] . Similar results were obtained in pigs exposed to HS after supplementation with vitamins C and E [23] .

The data indicated occurrence of tachycardia in all experimental groups of rabbits exposed to HS (Tables 2,5,8). The highest HR values were reported in IMO+HS rabbits, and the lowest values were reported in the IMO+HS + Vit. C or Vit. E-Se treated rabbits. During heat stress, both noradrenergic signaling and circulating catecholamine increase, leading to a global hyper-adrenergic state [24] . The tachycardia obtained during the current studies

could be attributed to the direct effect of heated blood on the cardiac pacemaker and the sympathetic and parasympathetic effects of the arterial baroreflexes or the hyperadrenergic state on the heart [25] . Elevation in blood temperature during heat stress was associated with cardiovascular responses including tachycardia in dogs [26,27] . In rabbits, exposure to hot humid environment caused significant increase in pulse rate [28] . Immobilization (IMO) may have augmented heat stress and thus induced tachycardia. Crestani *et al.* [29] reported tachycardia after exposure of rats to acute restraint stress. The attenuated tachycardia (Tables 5 and 8) could be attributed to the antioxidant properties of Vit. C and Vit. E-Se that alleviated the negative effect of stress by depressing the activity of central nervous system [30, 31].

4.2 The PCV :

In the current results, the PCV of HS and IMO+HS rabbits decreased, while that of Vit. C and vitamin E - Se treated rabbits slightly increased compared to the control rabbits (Tables 3, 6 and 9). Heat stress elevated blood temperature, and the erythrocyte osmotic fragility of erythrocytes was proportionally related to the blood temperature [32,33] due to high production of reactive free radicals [34] . The findings are in agreement with previous studies which reported haemocytopenia during exposure to hot environments in rabbits [6,35,36] and rats [37] . The slight increase in PCV obtained in Vit.C and Vit E-Se treated rabbits (Tables 6 and 9) is in accordance to previous studies in heat stressed rats, which attributed the increase to the role of Vit. C and vitamin E in alleviating harmful effect of heat stress on the erythrocytic membranes by scavenging oxidative free radicals and consequently decreasing haemolysis of erythrocytes [38] .

4.3 The Leukocytic Profile :

The TLC was decreased in most experimental groups of rabbits after the treatment compared to the control rabbit values (Tables 3, 6 and 9), followed by increased TLC, observed mainly in HS rabbits (Tables 6 and 9). Various stressors, including heat stress, are associated with high concentration of glucocorticoids and high environmental temperature causes multiple functional and metabolic changes in body tissues and cells including immune cells [18]. The leukopenia reported following heat stress in rabbits could be attributed to the presence of local chemotactic agents causing a shift of leukocytes to the reservoirs pools [39] .Ondruska *et al.* [36] reported significant leukopenia in rabbits after exposure to high ambient temperature. The increase in TLC observed in HS rabbits thereafter during the experiment compared to the treated rabbits (Tables 6 and 9) could be associated with the anti-corticosteroid activities of Vit. C and vitamin E which inhibit the release of leukocytes from their pools into the circulation [40] . The higher mean values of Ta and RH during day 3 of the trial (Table 1) may account for the remarkable leukopenia obtained in IMO+HS+Vit. E-Se (Table 9) compared to the IMO+HS+Vit. C treated rabbits (Table 6). The ability to regulate body temperature is influenced by environmental factors such as temperature, humidity and wind speed [41] **.** Furthermore , previous studies pointed to the ability of Vit. C and Vit. E to inhibit oxidative processes of lipids and lipoproteins in leukocytic cell membrane [42 , 43**].**

The current study indicated that the most pronounced changes in leukocytic profile were increase in lymphocyte percentage and decrease in neurophil percentage in rabbits exposed to IMO+HS compared to the control rabbit values (Tables 7 and 10). The lymphopenia and neutrophilia were more pronounced in HS and IMO+HS group rabbits compared to the other

experimental groups. Glucocorticoids produced during stress influence the lymphocytes subsets by redistributing them from peripheral blood , spleen and bone marrow to mesenteric lymph nodes and lymphoid tissues in and around the intestine [44] . Conversely, polymorphonuclear leukocytes released from the marrow [45], intravascular polymorphonuclear pools and the circulation [46] may account for the neutrophlia . Lymphopenia and neutrophilia were reported after acute heat stress in rabbits [47] . Simlar results were obtained in rats after exposure to restraint stress [48].

5. CONCLUSION

Immobilization and heat exposure constitute important factors that induce changes in homeostasis of mammals . The rabbit can be adopted as a suitable model for critical investigations of physiological responses . Immobilization can aggravate the negative effects of heat stress in a tropical environment with high radiation intensity . Vitamin C was more effective than Vitamin E–Se in alleviation of hyperthermia and maintenance of homeostasis and normal haematological parameters in the rabbit model .

Ethical disclaimer:

There were ethical issues that were addressed adequately according to the veterinary and institutional guidelines.

REFERENCES

1. Pisochi, A M , Pop A . The role of antioxidants in the chemistry of oxidative stress . A review . Eur. J. Med. Chem. 2015 ; 97 :55-74.

2. Pellegrino D . Antioxidants and cardiovascular risk factors. Diseases 2009 ; 4:1-9.

3. Chrousos G P . Stress and disorders of the stress system. Endocrinology. 2009 ; 5: 374-381.

4. Cazabon E P I , Rastogi R K , Laukner F B , Ali B A . Some haematological values in rabbits from subtropical Trinidade , West Indies. World Rabbit Sci. 2000 ; 8: 63-65.

5. Burnett N , Mathura K , Metivier K S, Holder R B , Brown G , Campbell M .An investigation into haematological and serum chemistry parameters of rabbits in Trinidad. World Rabbit Sci . 2006 ; 14: 175-187.

6. Okab, A B , El- Banna S G , Koriem A A . Influence of environmental temperatures on some physiological and biochemical parameters of New- Zealand rabbits. Slov. J. Anim. Sci. 2008 ; 41: 12-19.

7. Sahin E , Gumuslu S . Stress-dependent induction of protein oxidation, lipid peroxidation and anti-oxidants in peripheral tissues of rats: comparison of three stress models (immobilziation, cold and immobilization cold), Clin. Exp. Pharmacol. Physiol. 2007 ; 34: 425-431.

8. Akpinar D , Yargicoglu P , Derin N , Aslan M , Agar A . Effect of aminoguanidine on visual evoked potentials (VEPs), antioxidant status and lipid peroxidation in rats exposed to chronic restraint stress. Brain Res. 2007 ; 1186: 87-94.

9. Shirato K , Motohashi N , Tanihata J , Tachiyashiki K , Tomda A et al. Effects of two types of inactivity on the number of white blood cells in rats. Eur. J. Appl. Physiol. 2006 ; 98: 590-600.

10. Anand P , Rajakumar D , John W F , Balasubramanian T . Effects of oral administration of antioxidant taurine on haematological parameters in Wistar rats. Pak. J. Biol. Sci. 2010 ;13:785-793.

11. Sadau Y , Adelaiye A B , Magaji R A , Ayo J O , Isa A I et al. Ameliorative effects of selenium and vitamin E supplementation on some haematological parameters and red blood cell osmotic fragility in Wistar rats subjected to water immersion restraint stress. Bajopas . 2015 ; 8: 123-128.

12. Moazzam S , Hussain M M , Saleem S . Effects of ascorbic acid and alpha tocopherol on immune status of Sprauge Dawley rats exposed to chronic restraint stress . J. Ayub. Med. Coll. Abbotabad 2012 ; 24: 3-4 .

13. Ohta Y ,Kaida S , Chiba S , Tada M , Teruya A , Imai Y, Kawanishi M . Involvement of oxidative stress in increases in the serum levels of various enzymes and components in rats with water-immersion restraint stress. J. Clin. Biochem. Nutr. 2009 ; 45: 347-354.

14. Attia H F , , M.M. Soliman M M , Ismail T A . Protective effect of vitamin E and selenium on the liver, heart and aorta. J. Vet. Anat. 2012 ; 5: 17-29.

15. Al-Zafry S R , Medan M S . Effect of vitamin E and selenium complex on heat-stressed rabbits . SCVMJ,2012 XVII ; 2: 129-138.

16.Jain J . Haematologic techniques. In: Schalms Veterinary Haematology.4th Edition, (Edited by Jain N C) , Lea and Febiger, Philadelphia, USA., 1986 ; pp20-86.

17. SAS, SAS/STAT User Guide . SAS Institute , Inc. , Cary , N. Y. 2002 .

18. Kumar R , Kumar S , Ali M , Kumar A , Nath A , Lawrence K , J. K. Singh J K . Impact of stress on histology and biochemical parameters of liver and kidney of mice. Innov. J. Med. Health Sci. 2012 ; 2: 63-66.

19. Abdelatif A M , Saeed I H . Thermoregulation, heart rate and body weight as influenced by thyroid status and season in the domestic rabbit (*Lepus cuniculus*) . Middle-East. J. Sci. Res . 2009 ; 4: 310-319.

20. Flournoy W S , Wohl J S , Macintric D K . Heat stroke in dogs: pathophysiology and predisposing factors. Comp. Cont. Edu. Pract. Vet. 2003 ; 25: 410-418.

21. Johnson S I , McMichael M , White G . Heatstroke in small animal medicine: a clinical practice review. J. Vet. Emerg. Crit. Care. 2006 , 16: 112-119.

22. Seyrek K , Yenisey C , Serter M , Karg F et al. Effects of dietary vitamin C supplementation on some biochemical parameters of laying Japanese quails exposed to stress (34.8^{o}C). Revue Med.Vet. 2004 ; 156: 339-342.

23. Shenglin W , Yingcai L , Li Z , Yong J Z , Shouqun J . Effect of antiheat stressors on serum biochemical indexes and immunity of finishing pigs. Chinese J. Anim. Sci. 2003 ; 39: 11-12.

24. Rowell L B . Hyperthermia: a hyperadrenergic state. Hypertension . 1990 . 15:505–507.

25.Wilson T E , Tollund C , Yoshiga C C . Effects of heat and cold stress on central vascular pressure relationships during orthostasis in humans. J. Physiol. 2007 ; 585: 279-285.

26. Knochel J P , Reed G .Disorders of heat regulation . In: Clinical Disorders of Fluid and Electrolyte Metabolism.5th Edtion, Narins, R. G., Edited by Maxwell and Kleeman's. New York: McGraw-Hill, pp: 1549-1590 .1994 .

27. Romanucci M , Dell-Salda L. Pathophysiology and pathological findings of heatstroke in dogs. Vet. Med. Res.Reports 2013 ; 4: 1-9.

28. Fadare A O .Thermophysiological traits of California New Zealand White, Havana bucks and Palomino brown rabbits raised in humid tropics. J. Biol. Agric. Health. 2015 ; 4: 204-210.

29. Crestani C C , Tavares R F, Alves F H , Resstel L B , Correa F M . Effect of acute restraint stress on the tachycardic and bradycardic response of the baroreflex in rats. Stress 2010 ; 13: 61-72.

30. Najafpour A , Sadeghi G .Vitamin C pre-medication enhances the anaesthetic effect of ketamine-xylazine combination in the rat. Arch .Med . Sci . 2007 ; 4: 340-343.

31.Yanmaz L E , Dogan E , Okumus Z , Senocak M G , Prastiwi A et al. . Xylazine-ketamine anaesthesia following premedication of New Zealand White rabbits with vitamin C. Kafkas.Univ.Vet.Fak. Derg. 2015 ; 22: 115-118.

32. Oyewale J O . Changes in osmotic resistance of erythrocytes of cattle, pigs, rats and rabbits during variations in temperature and PH. J. Vet. Med. 1992 ; 39:98-104.

33. Oladele, S.B., J. O. Ayo, S. O. Ogundipe, and K. A. Esiero, 2003 . Seasonal and species variations in erythrocytes osmotic fragility of

indigenous poultry species in Zaria, Northern, Guinea Savannah zone of Nigeria. Bull. Anim. Health Prod. Afr., 51: 204-214.

34. Finkel, T. and J. Holbrook, 2000 . Oxidants ,oxidative stress and the biology of aging. Nature, 408: 239-247.

35. Okab A B , El Banna S G . Physiological and biochemical parameters in New Zealand White rabbits during spring and summer seasons . Egyp. J. Basic Appl. Physiol. 2003 ; 2:289-300 .

36. Ondruska L , Rafay J , Okab A B , Ayoub M A , Al-Haidary A A et al. . Influence of elevated ambient temperature upon some physiological measurements of New Zealand White rabbits. Veterinarni Medicina . 2011 ; 56: 180-186.

37. Abdul Wahab A ,Mabrouk M A , Ayo J O , Sulaiman A F , Muftau S et al. Effects of co-administration of antioxidants on erythrocyte osmotic fragility of Wistar rats during the hot- dry season. Eur. J. Sci. Res. 2010 ; 46: 73-79.

38 . Alhassan A W , Adenkola A Y , Yusuf A , Bauchi Z M , Saleh M I et al. Erythrocyte osmotic fragility of Wistar rats administered ascorbic acid during the hot-dry season. J. Cell Anim. Biol. 2010 ; 4: 29-33.

39. Joseph I M , Suthanthirarajan N , Namasivayam A . Effect of acute hest stress on certain immunogical parameters in Albino rats. Indian J. Physiol. Pharmacol. 1991 ; 35: 269-271

40. Whitehead C C , Keller T . An update on ascorbic acid in poultry WPSA. 2003 ; 59 :161-184.

41. Miyake Y . Pathophysiology of heat illness: Thermoregulation, risk factors and indicators of aggravation. JMAJ 2013 , 56: 167-173.

42. Kraust A , Roth H P , Kirchgessner M . Infuence of vitamin C, vitamin E and beta–carotene on the osmotic fragility and the primary antioxidant system of erythrocytes in zinc-deficient rats. Arch. Tierernahr.1997 ; 50 : 257-269.

43. Yavuz T, Delibas N , Yildrim B , Altuntas I , Cndri O . Vascular wall damage in rats induced by methidathion and ameliorating effect of vitamins E and C. Arch. Toxicol. 2004 ; 78:655-659.

44. Toft P , Svendsen P , Tonnesen E , Rasmussen J W and N. J. Christensen N J .Redistribution of lymphocytes after major surgical stress. Acta Anesthesiol.Scand. 1993 ; 37:245-249.

45. Burton J K , Kehrli S , Kapil, Horst R . Regulation of L-selectin and CD18 on bovine neutrophils by glucocorticoids: effects of cortisol and dexamethasne. J. Leukoc. Biol. 1995 ; 57: 317-325.

46. Nakagawa M , Terashima T , D'yachkova Y , Bondy G P , Hogg J C , VanEeden S F . Glucocorticoid-induced granulocytosis : contribution of marrow release and demargination of intravascular granulocytes. Circulation 1998 ; 98: 2307-2313.

47. Khalil H A , Yaseen M A , Hamdy A M . Behavioural activities, physiological body reactions, haematological parameters and hormonal profiles for bucks of New Zeland White and Baladi Red rabbits exposed to short term of high temperature. Asian. J. Poult. Sci. 2015 ; 9: 191-202.

48. Pereira-Figueiredo I , Carro L J , Munoz C , Sancho, Castellano O et al. 2015 . Sex differences in the effects of sertraline and stressors in rats previously exposed to restrain stress . JBiSE 2015 ; 8: 399-419.

Table1. The ambient temperature (T_a), relative humidity (RH) and wind speed (WS) during the experimental period .

Days	T_a(°C)			RH(%)	WS (Km/h)
	Maximum	Minimum	Mean	Mean	
Trial I	37.8	20.0	28.9	24.4	5.56
Trial II	30.6	13.0	21.8	25.6	9.26
Trial III	33.0	17.0	25	39.6	7.41

Table 2. Effects of acute heat stress (HS) and immobilization (IMO) on rectal temperature (Tr) and heart rate (HR) in male rabbits.

Parameter		Time (1 hour)	
		Initial	Final
Tr (°C)	Control	$38.62^{a} \pm 0.35$	$39.13^{a} \pm 0.21$
	HS	$38.50^{a} \pm 0.26$	$41.32^{d} \pm 0.52$***
	1MO + HS	$38.48^{a} \pm 0.26$	$42.00^{d} \pm 0.65$***
HR (Beats/min)	Control	$177.33^{a} \pm 13.54$	$176.33^{a} \pm 8.81$
	HS	$189.33^{a} \pm 18.70$	$230.00^{c} \pm 5.39$**
	1MO + HS	$181.33^{a} \pm 11.76$	$242.00^{b} \pm 6.51$*

For each parameter, mean values within the same column bearing different superscript are significantly different compared to the control .

***: p<0.05; **: p<0.01; ***: p<0.001.**

Table3. Effects of acute heat stress (HS) and immobilization (IMO) on packed cell volume (PCV) and total leukocyte count (TLC) in male rabbits.

Parameter	Time (1 hour)	
	Initial	Final

PCV (%)	Control	$32.50^{a} \pm 1.51$	$31.17^{a} \pm 1.48$
	HS	$33.17^{a} \pm 1.72$	$30.33^{a} \pm 0.82$
	1MO + HS	$30.50^{a} \pm 1.95$	$33.17^{a} \pm 1.31$
TLC ($X10^{3}/\mu L$)	Control	$7.25^{a} \pm 0.52$	$7.42^{a} \pm 0.92$
	HS	$7.33^{a} \pm 1.66$	$6.47^{a} \pm 1.20$
	1MO + HS	$6.75^{a} \pm 0.82$	$5.60^{a} \pm 2.32$

parameter , mean values within the same column bearing the same superscripts are not significantly different .

Table 4. Effects of acute heat stress (HS) and immobilization (IMO) on differential leukocyte (DLC) count in male rabbits.

Parameter		**Time (1hour)**	
		Initial	**Final**
Lymphocyte(%)	Control	$58.17^{a} \pm 3.37$	$59.33^{a} \pm 3.88$
	HS	$58.33^{a} \pm 3.83$	$59.17^{a} \pm 0.98$
	1MO+HS	$57.00^{a} \pm 4.34$	$58.67^{a} \pm 1.51$

	Control	34.83[a]±2.04	33.33[a]±3.39
Neutrophil(%)	HS	35.67[a]±4.41	34.33[a]±2.07
	1MO+HS	37.00[a]±5.06	35.50[a]±3.51
	Control	5.17[a]±0.75	4.50[a]±0.84
Monocyte(%)	HS	4.83[a]±0.41	4.50[a]±0.55
	1MO+HS	4.50[a]±1.05	3.83[a]±0.98
	Control	1.33[a]±0.82	1.50[a]±1.05
Eosinophil(%)	HS	0.83[a]±0.98	1.50[a]±1.05
	1MO+HS	1.33[a]±0.52	1.17[a]±0.75
	Control	0.50[a]±0.55	0.33[a]±0.52
Basophil(%)	HS	0.17[a]±0.41	0.17[a]±0.41
	1MO+HS	0.17[a]±0.41	0.67[a]±0.82

For each parameter, mean values within the same column bearing the same superscripts are not significantly different .

Table 5. Effects of acute heat stress (HS), immobilization (IMO) and administration of Vit.C on rectal temperature, and heart rate (HR) in male rabbits.

Parameter		Time (Hours)			
		0	2	24	48
Tr (°C)	Control	38.42^{a}±0.40	39.07^{a}±0.28	38.63^{a}±0.28	38.42^{a}±0.31
	HS	38.35^{a}±0.35	41.60^{d}±0.40***	38.85^{a}±0.19	38.90^{b}±0.23*
	1MO+HS	38.58^{a}±0.30	42.56^{d}±0.56***	39.43^{c}±0.34**	39.75^{c}±0.38**
	1MO+HS+Vit. C	38.22^{a}±0.65	41.98^{d}±0.47***	38.68^{a}±0.37	38.83^{a}±0.40
HR (Beats/min)	Control	195.33^{a}±4.85	191.83^{a}±3.32	200.00^{a}±5.73	198.33^{a}±3.10
	HS	195.00^{a}±4.68	193.33^{a}±6.01	248.67^{c}±6.70**	222.00^{a}±6.26
	1MO+HS	194.67^{a}±5.69	301.60^{c}±6.55**	259.00^{d}±5.64***	227.00^{a}±5.18
	1MO+HS+Vit. C	193.33^{a}±4.28	208.00^{a}±4.53	206.67^{a}±4.17	211.33^{a}±5.45

For each parameter, mean values within the same column bearing different superscripts are significantly different compared to the control.

***: p<0.05 **: p<0.01 *** :p<0.001.**

Table 6. Effects of acute heat stress (HS), immobilization (IMO) and administration of Vit.C on packed cell volume (PCV) and total leukocyte count (TLC) in male rabbits.

Parameter		Time (Hours)			
		0	**2**	**24**	**48**
PCV (%)	Control	$34.00^{a}\pm1.26$	$35.00^{a}\pm1.67$	$33.83^{a}\pm0.75$	$33.00^{a}\pm1.41$
	HS	$35.17^{a}\pm1.17$	$35.50^{a}\pm1.02$	$33.50^{a}\pm1.93$	$32.17^{a}\pm1.14$
	1MO+HS	$33.83^{a}\pm1.17$	$33.80^{a}\pm1.31$	$32.25^{a}\pm1.63$	$30.75^{b}\pm0.96$*
	MO+HS+Vit.C	$34.00^{a}\pm1.55$	$36.33^{a}\pm1.88$	$31.67^{a}\pm0.88$	$32.33^{a}\pm1.58$
TLC ($X10^{3}/\mu L$)	Control	$6.60^{a}\pm0.80$	$6.83^{a}\pm1.01$	$6.43^{a}\pm0.48$	$7.02^{a}\pm0.44$
	HS	$7.42^{a}\pm1.02$	$7.17^{a}\pm1.72$	$10.25^{c}\pm1.52$**	$9.00^{b}\pm1.07$*
	1MO+HS	$6.22^{a}\pm0.25$	$4.70^{c}\pm0.84$**	$7.13^{a}\pm1.93$	$7.50^{a}\pm1.78$
	MO+HS+Vit.C	$6.33^{a}\pm0.88$	$7.83^{a}\pm1.66$	$8.75^{b}\pm1.44$*	$8.33^{a}\pm1.25$

For each parameter, mean values within the same column bearing different superscripts are significantly different compared to the control .

***: p<0.05. ** : p<0.01.**

Table 7. Effects of acute heat stress (HS), immobilization (IMO) and administration of Vit.C on differential leukocyte count in male rabbits.

Parameter		Time(Hours)			
		0	2	24	48
Lymphocyte (%)	Control	57.67^{a}±2.80	57.67^{a}±3.01	57.83^{a}±2.14	57.17^{a}±2.32
	HS	57.33^{a}±1.75	57.83^{a}±2.32	61.67^{c}±1.86**	62.17^{b}±3.71*
	1MO+HS	58.00^{a}±1.03	59.00^{a}±4.12	52.25^{b}±3.86*	62.75^{b}±3.59*
	1MO+HS+Vit.C	57.67^{a}±1.75	56.50^{a}±4.23	60.83^{a}±3.76	61.00^{b}±2.19*
Neutrophil (%)	Control	36.17^{a}±1.33	36.67^{a}±1.51	36.50^{a}±1.64	36.83^{a}±1.17
	HS	35.67^{a}±1.21	36.17^{a}±3.19	32.17^{c}±1.33**	32.33^{b}±3.83*
	1MO+HS	34.83^{a}±0.75	34.60^{a}±5.27	43.00^{b}±5.42*	31.75^{c}±2.50**
	1MO+HS+Vit.C	36.17^{a}±1.60	38.00^{a}±5.06	32.67^{c}±1.03**	33.33^{c}±2.16**
Monocyte (%)	Control	4.17^{a}±0.75	4.17^{a}±0.98	4.17^{a}±0.75	4.00^{a}±0.89
	HS	5.50^{a}±0.55	3.83^{a}±1.72	4.83^{a}±0.75	4.67^{a}±0.82
	1MO+HS	5.33^{a}±0.82	4.80^{a}±0.84	4.00^{a}±0.82	4.45^{a}±0.96
	1MO+HS+Vit.C	5.17^{a}±0.75	4.50^{a}±1.05	4.00^{a}±0.89	4.67^{a}±0.52
Eosinophil (%)	Control	1.50^{a}±1.05	0.83^{a}±0.75	1.33^{a}±0.82	1.33^{a}±0.52
	HS	1.00^{a}±0.63	1.50^{a}±1.05	0.83^{a}±0.75	0.67^{a}±0.82
	1MO+HS	0.83^{a}±0.98	1.60^{a}±1.14	1.25^{a}±1.50	0.50^{b}±0.58*
	1MO+HS+Vit.C	1.00^{a}±0.63	1.00^{a}±0.89	0.50^{a}±0.50	1.17^{a}±0.75
Basophil (%)	Control	0.50^{a}±0.55	0.50^{a}±0.55	0.17^{a}±0.41	0.50^{a}±0.55
	HS	0.50^{a}±0.55	0.00^{a}±0.00	0.50^{a}±0.55	0.17^{a}±0.41
	1MO+HS	0.17^{a}±0.41	0.00^{a}±0.00	0.25^{a}±0.50	0.25^{a}±0.50
	1MO+HS+Vit.C	0.00^{a}±0.05	0.00^{a}±0.00	0.33^{a}±0.52	0.17^{a}±0.41

For each parameter, mean values within the same column bearing different superscripts are significantly different compared to the control.

***: p<0.05 ** : p<0.01.**

Table 8. Effects of acute heat stress (HS), immobilization (IMO) and administration of Vit.E-Selenium on rectal temperature (Tr) and heart rate (HR) in male rabbits.

Parameter		Time (Hours)			
		0	**2**	**24**	**48**
Tr(°C)	Control	$38.70^{a}\pm0.36$	$39.05^{a}\pm0.39$	$39.03^{a}\pm0.40$	$39.08^{a}\pm0.41$
	HS	$38.55^{a}\pm0.48$	$42.20^{d}\pm0.52$***	$39.97^{a}\pm0.64$	$39.30^{a}\pm0.48$
	1MO+HS	$38.42^{a}\pm0.27$	$42.64^{d}\pm0.38$***	$39.46^{a}\pm0.36$	$39.70^{b}\pm0.46$*
	1MO+HS+Vit.E+Se	$38.80^{a}\pm0.71$	$41.35^{c}\pm1.47$**	$39.30^{a}\pm0.22$	$39.43^{a}\pm0.13$
HR (Beats/min)	Control	$190.00^{a}\pm5.58$	$211.33^{a}\pm4.69$	$206.67^{a}\pm5.27$	$207.33^{a}\pm5.88$
	HS	$191.33^{a}\pm4.45$	$218.00^{a}\pm3.15$	$225.33^{a}\pm5.93$	$209.33^{a}\pm5.64$
	1MO+HS	$203.67^{a}\pm4.28$	$298.60^{c}\pm5.46$**	$279.20^{c}\pm5.49$**	$248.00^{c}\pm6.68$**
	1MO+HS+Vit.E+Se	$196.67^{a}\pm5.53$	$247.33^{a}\pm5.12$	$229.00^{a}\pm6.18$	$206.00^{a}\pm5.07$

For each parameter, mean values within the same column bearing different superscripts are significantly different compared to the control.

***: p<0.05 **: p<0.01 ***: P<0.001.**

Table 9. Effects of acute heat stress (HS), immobilization(IMO) and administration ofVit.E-Selenium on packed cell volum (PCV) and total leukocyte (TLC) in male rabbits.

Parameter		Time (Hours)			
		0	2	24	48
PCV (%)	Control	35.67^{a}±1.03	34.39^{a}±1.16	34.50^{a}±1.27	31.17^{a}±0.66
	HS	36.50^{a}±1.39	34.18^{a}±1.60	32.83^{a}±1.06	31.33^{a}±1.25
	1MO+HS	35.67^{a}±1.03	33.58^{a}±1.77	31.00^{a}±0.92	30.40^{a}±1.05
	1MO+HS+Vit.E+Se	34.33^{a}±1.97	36.96^{a}±1.38	33.00^{a}±0.83	31.75^{a}±0.50
TLC ($X10^{3}/\mu L$)	Control	7.75^{a}±0.42	6.50^{a}±0.84	7.08^{a}±1.32	7.50^{a}±1.22
	HS	7.33^{a}±1.21	4.50^{c}±0.77**	9.17^{a}±1.75	6.50^{a}±0.45
	1MO+HS	7.67^{a}±0.92	4.90^{a}±1.82	7.30^{a}±0.84	5.10^{c}±0.74**
	1MO+HS+Vit.E+Se	7.25^{a}±1.60	5.38^{a}±1.25	6.00^{a}±1.08	6.25^{a}±0.50

For each parameter, mean values within the same column bearing different superscripts are significantly different compared to the control.

**** : P <0.01.**

Table10. Effects of acute heat stress (HS), immobilization (IMO) and administration of Vit.E-Selenium on differential leukocyte count in male rabbits.

Parameter		Time (Hours)			
		0	2	24	48
Lymphocyte (%)	Control	59.67^{a}±1.37	57.17^{a}±2.79	61.33^{a}±2.16	58.83^{a}±3.76
	HS	58.07^{a}±1.83	52.00^{b}±3.10*	60.50^{a}±1.76	58.00^{a}±4.56
	1MO+HS	61.67^{a}±0.08	52.60^{b}±3.10*	58.00^{c}±1.41**	56.80^{a}±2.24
	1MO+HS+Vit.E+Se	60.67^{a}±0.11	59.00^{a}±1.15	60.25^{a}±2.22	59.00^{a}±1.41
Neutrophil (%)	Control	34.67^{a}±2.42	37.83^{a}±3.31	33.33^{a}±2.16	35.33^{a}±4.63
	HS	32.17^{a}±1.47	45.38^{c}±4.45**	33.00^{a}±1.67	36.17^{a}±5.64
	1MO+HS	31.83^{a}±1.17	42.40^{c}±12.95**	35.40^{a}±0.71	36.80^{a}±5.17
	1MO+HS+Vit.E+Se	32.67^{a}±1.37	32.75^{a}±6.13	33.75^{a}±2.22	34.50^{a}±1.91

Monocyte (%)	Control	4.33^{a}±0.52	4.67^{a}±0.82	4.67^{a}±0.82	5.00^{a}±1.26
	HS	5.00^{a}±0.89	2.50^{c}±0.84**	4.50^{a}±0.55	4.50^{a}±1.05
	1MO+HS	5.50^{a}±0.55	1.20^{a}±2.06	5.00^{a}±1.41	4.80^{a}±0.45
	1MO+HS+Vit.E+Se	5.17^{a}±0.75	4.75^{a}±0.50	5.00^{a}±0.82	5.00^{a}±0.85
Eosinophil (%)	Control	1.17^{a}±0.98	0.83^{a}±0.75	0.67^{a}±0.52	0.50^{a}±0.84
	HS	2.17^{a}±0.41	0.00^{b}±0.00*	1.67^{a}±0.52	1.17^{a}±0.75
	1MO+HS	1.00^{a}±1.10	0.20^{a}±1.22	1.80^{b}±1.14*	1.20^{a}±1.10
	1MO+HS+Vit.E+Se	1.00^{a}±0.89	0.75^{a}±0.50	0.75^{a}±0.50	1.50^{a}±0.58
Basophil (%)	Control	0.33^{a}±0.52	0.20^{a}±0.04	0.33^{a}±0.52	0.17^{a}±0.41
	HS	0.17^{a}±0.41	0.33^{a}±0.52	0.33^{a}±0.52	0.33^{a}±0.52
	1MO+HS	0.17^{a}±0.41	0.92^{a}±2.06	0.20^{a}±0.10	0.40^{a}±0.55
	1MO+HS+Vit.E+Se	0.00^{a}±0.15	0.25^{a}±0.50	0.25^{a}±0.50	0.10^{a}±0.05

For each parameter, mean values within the same column bearing different superscripts are significantly different compared to the control.

***: p<0.05 ** : p<0.01.**