

1 **Effects of Immobilization and Heat Stress and Supplementation of**
2 **Antioxidants on Thermoregulation and Haematological Responses**
3 **in Male Rabbits (*Oryctolagus cuniculus*)**
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9 **ABSTRACT**

10 **Backgoud and Objectives** : Immobilization stress may induce negative
11 effects on physical and physiological activities of humans and animals. Thermal
12 load also influences the wellbeing and health of mammals , particularly under
13 tropical conditions . This study aimed to evaluate the responses to immobilization
14 (IMO) and acute heat stress (HS) in a rabbit model . The potential protective
15 effects of administration of antioxidants on IMO and acute heat stress (HS) were
16 also assessed . **Materials and Methods** : Sixty six male rabbits (mean BW
17 1582±28g) were used in three trials to investigate the effects of HS , IMO+HS and
18 administration of vitamin C (IMO+HS +Vit C) or vitamin E-selenium (IMO+HS
19 +Vitamin E-Se). Immobilization was performed by fixing the animals in a specially
20 designed box ; HS was induced by exposing rabbits to direct solar radiation (370
21 W/m²) for 1 hour (trial 1) and 2 hrs (trials 2 and 3). The body weight (BW) ,
22 rectal temperature (Tr) and heart rate (HR) were monitored and venous blood
23 samples were collected before the beginning of the trial and then at 2, 24 and 48 hrs
24 after the end of the trial. The packed cell volume (PCV) , total leukocytes count
25 (TLC) and differential leukocytes count (DLC) were determined . **Results** :In trial-

26 I, 18 rabbits were randomly assigned to 3 groups of 6 each (control, HS and
27 IMO+HS). HS rabbits showed higher values of HR ($P<0.01$) compared to
28 IMO+HS rabbits . In trial -II , 24 rabbits were assigned to 4 groups comprising
29 control, HS, IMO+HS (received 2 doses of normal saline) and IMO+HS +Vit. C
30 (received 2 doses of 300 mg/kg/BW each Vit.C s/c). IMO+HS animals had higher
31 responses compared to HS, as evidenced by significantly ($P<0.01$) higher values of
32 Tr and HR. Administration of Vit. C decreased Tr, and maintained HR and
33 haematological parameters relatively constant. In trial 3, 24 rabbits were assigned to
34 4 groups comprising control(received 2 doses of normal saline s/c) , HS, IMO+HS
35 and IMO+HS +Vit. E-Se (received 2 doses 100 mg/kg/BW each Vit E-Se s/c). The
36 responses of animals to IMO+HS were greater compared to HS alone. IMO+HS
37 significantly ($P<0.001$) increased Tr and HR. Furthermore, IMO+HS rabbits
38 showed significant ($P<0.001$) decreases in PCV and TLC after 48hrs and 24 hrs,
39 repectively, compared to the values of control rabbits. Administration of Vit.E-Se
40 decreased Tr, HR and maintained haematological parameters relatively constant.
41 **Conclusion** :The study concluded that immobilization aggravated the negative
42 effects of heat stress, while Vit. C was more effective than Vit.E-Se in alleviation
43 of hyperthermia and maintaining normal haematological parameters in rabbits .

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

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49 1. INTRODUCTION

50 Stress is associated with increased incidence of morbidity and mortality rates
51 in animals and humans . The induced oxidative stress influences body

52 homeostasis [1] which plays a major role in prevalence of several health
53 problems that include cardiovascular diseases [2] , hypertension, and other
54 metabolic disorders [3]. Exposure of rabbits to high environmental
55 temperature caused disturbances in blood parameters, enzymatic reactions
56 and hormonal secretions [4-6] . Under certain circumstances **heat stress**(HS)
57 could be associated with **immobilization (IMO)** stress. IMO has been
58 considered as an acceptable protocol for physical and psychological stress in
59 mammals [7,8] . It could be associated with several physiological and
60 haematological changes involving leukocyte and erythrocytes [9 -11].

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

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72 **2. MATERIALS AND METHODS**

73 **2.1 Animals, Housing, Feeding and Management**

74 Sixty six (66) mature male rabbits with an average BW of 1582±28g were used
75 **. Animals** were kept in the animal house at the **Department of Physiology** in
76 individual cages and were allowed to adapt to the experimental procedures for
77 two weeks. During the adaptation period, animals were given access to food

78 and tap water *ad libitum*. Animals were given fresh lucerne (*Medicago sativa*)
79 and a rich source of starch (Sorghum grains). All animals were given a
80 prophylactic dose of anthelmintic injection (Ivermectin 0.02 ml/kg BW) and
81 antibacterial injection (Oxytetracycline: 7.5 mg/kg BW).

82 **2.2 Immobilization of animals**

83 Immobilizations stress was induced using a specially designed wood box (102 x
84 32 x 22 cm). The box was divided into 6 individual chambers and supplied with
85 horizontal tape to restrain the animals .During experimental periods , animals
86 were placed inside the immobilization device and fixed gently, with their heads
87 outside the chambers .

88 **2.3 Thermoregulation, Heart Rate (HR) and Body Weight (BW)**

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

93 **2.4 Haematological Parameters**

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

97 **2.5 Statistical Analysis**

98 The data were analysed using statistical analysis software [17] . One-way
99 ANOVA test according to complete randomized design(CRD) was used . The
100 difference between means was separated by least significant difference (LSD)
101 test. The results were presented as mean±SD and the P<0.05 was considered
102 statistically significant.

103 **2.6 Experimental Design**

104 In trial-I , 18 rabbits were assigned to three groups with equal numbers :
105 control group rabbits were on free movement under shade, heat stress
106 group(HS) rabbits were on free movement and subjected to heat stress by
107 exposure to direct solar radiation for 1hour, heat stressed and immobilized
108 (HS+IMO) rabbits were subjected to the specified treatments for 1hour. In
109 trial-II , 24 rabbits were randomly assigned to 4 groups with equal numbers :
110 control rabbits were on free movement under shade, heat stressed HS rabbits
111 were on free movement and subjected to heat stress for 2hrs, HS+IMO animals
112 were injected with normal saline and then subjected to HS+IMO stress for 2
113 hrs, and HS+IMO+Vit.C treated, HS+IMO+Vit.C rabbits received 2 doses of
114 300mg(s/c) of Vit. C/kg (Troy Laboratories PTY, Ltd , Australia) .The first
115 dose was injected one week prior to the experiment and the second dose was
116 injected immediately before the animals were subjected to heat
117 stress+immobilization for 2 hrs. The initial baseline values for
118 thermoregulation were obtained and blood samples were taken before the
119 beginning of the trial and then at 2, 24 and 48hrs after the end of the treatments
120 . In trial-III , 24 rabbits were randomly assigned to four groups with equal
121 numbers : control rabbits were on free movement under shade, heat stressed
122 (HS) rabbits were on free movement and subjected to heat stress for 2hrs, heat
123 stressed +immobilized (HS+IMO) rabbits were injected with normal saline and
124 then subjected to HS+IMO stress for 2hrs , and heat stressed, immobilized and
125 Vit.E+Se(IMO+HS+Vit.E-Se) rabbits were pre-administered two doses of
126 100mg/kg Vit.E–Se (Fravet Laboratories B.V., Netherlands) each s/c . The first
127 dose was injected one week before the treatment while the second dose was
128 injected immediately before subjecting animals to heat stress and
129 immobilization for 2hrs. For all trials, the initial baseline values for
130 thermoregulation were obtained and blood samples were taken before the onset

131 of the experiment and then at 2, 24 and 48 hrs after the end of exposure to
132 treatments .

133 **2. RESULTS**

134 **2.1 Effects of Acute Heat Stress (HS) and Immobilization (IMO) for One** 135 **Hour**

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140 **2.1.1 Climatic conditions**

141 The data of ambient temperature(T_a), relative humidity (RH) and wind speed
142 (WS) during the experimental period (November and December, 2014) are
143 presented in Table 1.

144 **2.1.2 Rectal Temperature(T_r) and Heart Rate (HR)**

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

150 **2.1.3 Packed Cell Volume (PCV) and Total Leukocyte Count (TLC)**

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

158 2.1.4 Differential Leukocyte Count (DLC)

159 The effects of HS and IMO+HS on DLC are illustrated in Table 4. The data
160 indicate non-significant difference in the ratios of lymphocytes and neutrophils
161 of HS and IMO+HS rabbits compared to respective control group values. The
162 monocyte ratio was non-significantly different between HS and IMO+HS
163 rabbits compared to the mean value of the control group rabbits. However, the
164 data showed that in IMO+HS rabbits, the monocyte ratio was slightly
165 decreased compared to the control rabbits. The eosinophil ratio of IMO+HS
166 rabbits was slightly decreased after the treatments compared to the value of the
167 control group rabbits. The basophil ratio was slightly increased in IMO+HS
168 rabbits compared to the respective values of the control rabbits.

169 2.2 Effects of Heat Stress, Immobilization and Administration of Vitamin
170 C.**171 2.2.1 Rectal temperature (Tr) and heart rate (HR)**

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

183 2.2.2 Packed Cell Volume (PCV) and Total Leukocyte count (TLC)

184 The effects of HS, IMO+HS and IMO+HS + Vit. C on PCV and TLC are
185 presented in Table 6. The PCV was significantly ($P<0.05$) lower in IMO+HS
186 rabbits after 48hrs compared to the respective control value . The pattern
187 indicates that the PCV of IMO+HS + Vit. C treated rabbits was slightly higher
188 after 2hrs, and then slightly lower after 24hrs compared to the control group at
189 the same time points. The TLC was significantly ($P<0.01$) decreased in
190 IMO+HS rabbits after 2hrs, significantly increased in HS rabbits after 24hrs
191 ($P<0.01$) and 48hrs ($P<0.05$), and in IMO+HS + Vit. C treated rabbits after
192 24hrs ($P<0.05$) compared to the control group values . The TLC was lowest in
193 IMO+HS rabbits throughout the experimental period, and Vit. C administration
194 relatively maintained the TLC in rabbits.

195 **2.2.3 Differential Leukocyte Count (DLC)**

196 The effects of HS, IMO+HS and IMO+HS + Vit. C on DLC in rabbits are
197 presented in Table 7. The lymphocyte ratio was significantly increased in HS
198 rabbits after 24hrs ($P<0.01$) and 48hr ($P<0.05$). In IMO+HS rabbits, a
199 significant ($P<0.05$) decrease was obtained after 24hrs , however , a significant
200 ($P<0.05$) increase was obtained in the same experimental group after 48hrs.
201 Also there was a significant ($P<0.05$) increase in lymphocyte ratio of
202 IMO+HS + Vit. C rabbits after 48hrs compared to the respective control values.
203 There was a significant decrease in neutrophil ratio in HS rabbits after 24hrs
204 ($P<0.01$) and 48hrs ($P<0.05$). In IMO+HS rabbits, the ratio was significantly
205 ($P<0.05$) increased after 24hrs, however, it was significantly ($P<0.01$)
206 decreased after 48hrs. In IMO+HS + Vit. C rabbit, a significant ($P<0.01$)
207 decrease was obtained after 24 and 48 hrs compared to the respective control
208 values. The monocyte ratio was slightly decreased in HS rabbits after 2hrs
209 compared to the respective mean value of control rabbits. The results indicate
210 that the eosinophil ratio was significantly ($P<0.05$) decreased in IMO+HS

211 rabbits after 48hrs compared to the control rabbits. The basophil ratio decreased
212 significantly ($P<0.05$) in HS rabbits after 2 hrs compared to the respective
213 control group value.

214 **2.3 Effect of Heat Stress, Immobilization and Administration of Vit. E–Se**

215 **2.3.1 Rectal Temperature (Tr) and Heart Rate (HR)**

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

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229 **2.3.2 Packed Cell Volume (PCV) and Total Leukocyte count (TLC)**

230 The effects of HS, IMO+HS and IMO+HS+Vit. E-Se on PCV and TLC in
231 male rabbits are presented in Table 9. The pattern indicates that the PCV of
232 HS and IMO+HS rabbits was slightly decreased after 2 and 24 hrs and the
233 PCV of IMO+HS+Vit. E-Se rabbits was slightly increased after 2hrs
234 compared to the respective control group values. The PCV of IMO+HS
235 rabbits maintained the lowest value throughout the experimental period. The
236 TLC decreased significantly ($P<0.01$) after 2hrs and then increased after
237 24hrs in HS rabbits. A non-significant decrease was obtained in IMO+HS

238 rabbits after 2hrs and a significant ($P<0.01$) decrease was reported after
239 48hrs. In IMO+HS+Vit. E-Se rabbits, TLC was decreased after 2hrs, and
240 the values remained lower after 24 hrs and 48 hrs compared to the respective
241 control group values. In IMO+HS +Vit. E-Se rabbits, TLC values were
242 relatively maintained compared to the other experimental groups .

2.3.3 Differential Leukocyte Count (DLC)

244 Table 10 shows the effect of HS, IMO+HS and IMO+HS+Vit. E-Se on DLC
245 . The lymphocyte ratio decreased significantly in HS rabbits after 2hrs
246 ($P<0.05$), and in IMO+HS rabbits after 2hrs ($P<0.05$) and 24hrs ($P<0.01$). The
247 lymphocyte ratio was lowest in IMO+HS rabbits throughout most of the
248 experimental period. Administration of Vit. E-Se alleviated the lymphopenia
249 induced by IMO+HS. There was a significant ($P<0.01$) increase in neutrophil
250 ratio of HS rabbits after 2hrs. The data also indicate a significant ($P<0.01$)
251 increase in neutrophil ratio of IMO+HS rabbits after 2hrs compared to the
252 respective control group values . Administration of Vit. E-Se ameliorated the
253 neutrophilia induced by IMO+HS. The monocyte ratio was significantly
254 ($P<0.01$) decreased in HS rabbits and non-significantly decreased in IMO+HS
255 rabbits after 2hrs. Administration of Vit. E-Se maintained the monocyte ratio
256 induced by IMO+HS. The eosinophil ratio was significantly ($P<0.05$)
257 decreased in HS rabbits after 2hrs, followed by non-significant increase after 24
258 hrs and 48 hrs. In IMO+HS rabbits, the eosinophil ratio was significantly
259 increased ($P<0.05$) after 24hrs compared to the control value. The pattern
260 indicates that the eosinophil ratio of IMO+HS rabbits decreased after 2hrs, and
261 increased after 48hrs. The eosinophil ratio increased non-significantly in
262 IMO+HS+Vit. E-Se rabbits after 48hrs. However, administration of Vit. E-Se
263 maintained the eosinophil ratio relatively constant after 2hrs and 24hrs. The

264 **basophil ratio** decreased in IMO+HS rabbits after 24 hrs. Administration of
265 Vit. E-Se slightly reversed the change in basophil ratio induced by IMO+HS.

266 **4.DISCUSSION**

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

283 The data indicated occurrence of tachycardia in all experimental groups of
284 rabbits exposed to HS (Tables 2,5,8). The highest HR values were reported
285 in IMO+HS rabbits, and the lowest values were reported in the IMO+HS +
286 Vit. C or Vit. E-Se treated rabbits. During heat stress, both noradrenergic
287 signaling and circulating catecholamine increase, leading to a global hyper-
288 adrenergic state [24]. The tachycardia obtained during the current studies
289 could be attributed to the direct effect of heated blood on the cardiac
290 pacemaker and the sympathetic and parasympathetic effects of the arterial

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

301 In the current results, the PCV of HS and IMO+HS rabbits decreased, while
302 that of Vit. C and vitamin E - Se treated rabbits slightly increased compared
303 to the control rabbits (Tables 3, 6 and 9). Heat stress elevated blood
304 temperature, and the erythrocyte osmotic fragility of erythrocytes was
305 proportionally related to the blood temperature [32,33] due to high
306 production of reactive free radicals [34]. The findings are in agreement
307 with previous studies which reported haemocytopenia during exposure to
308 hot environments in rabbits [6,35,36] and rats [37]. The slight increase in
309 PCV obtained in Vit.C and Vit E-Se treated rabbits (Tables 6 and 9) is in
310 accordance to previous studies in heat stressed rats, which attributed the
311 increase to the role of Vit. C and vitamin E in alleviating harmful effect of
312 heat stress on the erythrocytic membranes by scavenging oxidative free
313 radicals and consequently decreasing haemolysis of erythrocytes [38].
314 The TLC was decreased in most experimental groups of rabbits after the
315 treatment compared to the control rabbit values (Tables 3, 6 and 9), followed
316 by increased TLC, observed mainly in HS rabbits (Tables 6 and 9). Various
317 stressors, including heat stress, are associated with high concentration of

318 glucocorticoids and high environmental temperature causes multiple
319 functional and metabolic changes in body tissues and cells including
320 immune cells [18]. The leukopenia reported following heat stress in rabbits
321 could be attributed to the presence of local chemotactic agents causing a
322 shift of leukocytes to the reservoirs pools [39]. Ondruska *et al.* [36]
323 reported significant leukopenia in rabbits after exposure to high ambient
324 temperature. The increase in TLC observed in HS rabbits thereafter during
325 the experiment compared to the treated rabbits (Tables 6 and 9) could be
326 associated with the anti-corticosteroid activities of Vit. C and vitamin E
327 which inhibit the release of leukocytes from their pools into the circulation
328 [40]. The higher mean values of Ta and relative humidity (RH) during day
329 3 of the trial (Table 1) may account for the remarkable leukopenia obtained
330 in IMO+HS+Vit. E-Se (Table 9) compared to the IMO+HS+Vit. C treated
331 rabbits (Table 6). The ability to regulate body temperature is influenced by
332 environmental factors such as temperature, humidity and wind speed [41].
333 Furthermore, previous studies pointed to the ability of Vit. C and Vit. E to
334 inhibit oxidative processes of lipids and lipoproteins in leukocytic cell
335 membrane [42, 43].

336 The current study indicated that the most pronounced changes in leukocytic
337 profile were increase in lymphocyte ratio and decrease in neutrophil ratio
338 in rabbits exposed to IMO+HS compared to the control rabbit values (Tables
339 7 and 10). The lymphopenia and neutrophilia were more pronounced in HS
340 and IMO+HS group rabbits compared to the other experimental groups.
341 Glucocorticoids produced during stress influence the lymphocytes subsets
342 by redistributing them from peripheral blood, spleen and bone marrow to
343 mesenteric lymph nodes and lymphoid tissues in and around the intestine
344 [44]. Conversely, polymorphonuclear leukocytes released from the marrow

345 [45], intravascular polymorphonuclear pools and the circulation [46] may
346 account for the neutrophilia . Lymphopenia and neutrophilia were reported
347 after acute heat stress in rabbits [47] . Similar results were obtained in rats
348 after exposure to restraint stress [48].

349

350 5. CONCLUSION

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

358

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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

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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 ± 0.35	39.13 ^a ± 0.21
	HS	38.50 ^a ± 0.26	41.32 ^d ± 0.52
	1MO + HS	38.48 ^a ± 0.26	42.00 ^d ± 0.65
HR (Beats/min)	Control	177.33 ^a ± 13.54	176.33 ^a ± 8.81
	HS	189.33 ^a ± 18.70	230.00 ^c ± 5.39
	1MO + HS	181.33 ^a ± 11.76	242.00 ^b ± 6.51

For each parameter, means within the same column bearing different superscript are significantly different compared to the control.
a,b: Significant at p<0.05; a,c: Significant at p<0.01; a,d: Significant at p<0.001.

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Table3. Effects of acute heat stress (HS) and immobilization (IMO) on packed cell

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volume (PCV) and total leukocyte count (TLC) in male rabbits.

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Parameter		Time (1 hour)	
		Initial	Final
PCV (%)	Control	32.50 ^a ± 1.51	31.17 ^a ± 1.48
	HS	33.17 ^a ± 1.72	30.33 ^a ± 0.82
	1MO + HS	30.50 ^a ± 1.95	33.17 ^a ± 1.31
TLC (X10 ³ /μL)	Control	7.25 ^a ± 0.52	7.42 ^a ± 0.92
	HS	7.33 ^a ± 1.66	6.47 ^a ± 1.20
	1MO + HS	6.75 ^a ± 0.82	5.60 ^a ± 2.32

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parameter, means within the same column bearing the same superscripts are not significantly different compared to the control.

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a,a: Not significant.

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

Parameter		Time (1hour)	
		Initial	Final
Lymphocyte(%)	Control	58.17 ^a ±3.37	59.33 ^a ±3.88
	HS	58.33 ^a ±3.83	59.17 ^a ±0.98
	1MO+HS	57.00 ^a ±4.34	58.67 ^a ±1.51
Neutrophil(%)	Control	34.83 ^a ±2.04	33.33 ^a ±3.39
	HS	35.67 ^a ±4.41	34.33 ^a ±2.07
	1MO+HS	37.00 ^a ±5.06	35.50 ^a ±3.51
Monocyte(%)	Control	5.17 ^a ±0.75	4.50 ^a ±0.84
	HS	4.83 ^a ±0.41	4.50 ^a ±0.55
	1MO+HS	4.50 ^a ±1.05	3.83 ^a ±0.98
Eosinophil(%)	Control	1.33 ^a ±0.82	1.50 ^a ±1.05
	HS	0.83 ^a ±0.98	1.50 ^a ±1.05
	1MO+HS	1.33 ^a ±0.52	1.17 ^a ±0.75
Basophil(%)	Control	0.50 ^a ±0.55	0.33 ^a ±0.52
	HS	0.17 ^a ±0.41	0.17 ^a ±0.41
	1MO+HS	0.17 ^a ±0.41	0.67 ^a ±0.82

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For each parameter, means within the same column bearing the same super scripts are significantly not different compared to the control.

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a,a:Not significant.

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582 Table 5.E Effects of acute heat stress (HS), immobilization (IMO) and administration of
 583 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

584 For each parameter, means within the same column bearing different superscripts are significantly
 585 different compared to the control.

586 a,b: Significant at p<0.05.; a,c: Significant at p<0.01.; a,d: Significant at p<0.001.

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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 ±1.26	35.00 ^a ±1.67	33.83 ^a ±0.75	33.00 ^a ±1.41
	HS	35.17 ^a ±1.17	35.50 ^a ±1.02	33.50 ^a ±1.93	32.17 ^a ±1.14
	1MO+HSMO+HS+Vit.C	33.83 ^a ±1.17	33.80 ^a ±1.31	32.25 ^a ±1.63	30.75 ^b ±0.96
		34.00 ^a ±1.55	36.33 ^a ±1.88	31.67 ^a ±0.88	32.33 ^a ±1.58
TLC (X10 ³ /μL)	Control	6.60 ^a ±0.80	6.83 ^a ±1.01	6.43 ^a ±0.48	7.02 ^a ±0.44
	HS	7.42 ^a ±1.02	7.17 ^a ±1.72	10.25 ^c ±1.52	9.00 ^b ±1.07
	1MO+HS	6.22 ^a ±0.25	4.70 ^c ±0.84	7.13 ^a ±1.93	7.50 ^a ±1.78
	MO+HS+Vit.C	6.33 ^a ±0.88	7.83 ^a ±1.66	8.75 ^b ±1.44	8.33 ^a ±1.25

602 For each parameter, means within the same column bearing different superscripts are significantly
603 different compared to the control.
604 a,b: Significant at p<0.05.; a,c: Significant at p<0.01.

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622 **Table 7. Effects of acute heat stress (HS), immobilization (IMO) and administration of**
623 **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 ^b ±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

624 For each parameter, means within the same column bearing different superscripts are significantly
 625 different compared to the control.

626 a,a:Not significant.; a,b: Significant at $p<0.05$.; a,c: Significant at $p<0.01$.

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

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Parameter	Time (Hours)				
	0	2	24	48	
Tr(°C)	Control	38.70 ^a ±0.36	39.05 ^a ±0.39	39.03 ^a ±0.40	39.08 ^a ±0.41
	HS	38.55 ^a ±0.48	42.20 ^d ±0.52	39.97 ^a ±0.64	39.30 ^a ±0.48
	1MO+HS	38.42 ^a ±0.27	42.64 ^d ±0.38	39.46 ^a ±0.36	39.70 ^b ±0.46
	1MO+HS+Vit.E+Se	38.80 ^a ±0.71	41.35 ^c ±1.47	39.30 ^a ±0.22	39.43 ^a ±0.13
HR (Beats/min)	Control	190.00 ^a ±5.58	211.33 ^a ±4.69	206.67 ^a ±5.27	207.33 ^a ±5.88
	HS	191.33 ^a ±4.45	218.00 ^a ±3.15	225.33 ^a ±5.93	209.33 ^a ±5.64
	1MO+HS	203.67 ^a ±4.28	298.60 ^c ±5.46	279.20 ^c ±5.49	248.00 ^c ±6.68
	1MO+HS+Vit.E+Se	196.67 ^a ±5.53	247.33 ^a ±5.12	229.00 ^a ±6.18	206.00 ^a ±5.07

634 For each parameter, means within the same column bearing different superscripts are significantly
 635 different compared to the control.

636 a,b: Significant at $p<0.05$.; a,c: Significant at $p<0.01$.; a,d:Significant at $P<0.001$.

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644 Table 9. Effects of acute heat stress (HS), immobilization(IMO) and administration of Vit.E-
645 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 /μL)	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

646 For each parameter, means within the same column bearing different superscripts are significantly
647 different compared to the control.

648 a,c: Significant at P <0.01.

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650 **Table10. Effects of acute heat stress (HS), immobilization (IMO) and administration of**
 651 **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

652 For each parameter, means within the same column bearing different superscripts are significantly
 653 different compared to the control.

654 a,b: Significant at p<0.05.; a,c: Significant at p<0.01.

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