

Effect of Mint (*Mentha Longifolia*) Leaves Extraction on Some Physiological and Hormonal Traits of New Zealand White Rabbit Does During Summer

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Abstract: 25 New Zealand White rabbit does used in this study, were assigned randomly into two groups. In group one, 10 rabbit does were served as a control, while in group two 15 rabbit does, were daily gulped 8 ml of mint leaves extract (3 g/200ml boiled water) for 14 days. Traits used for the evaluation of the treatment effect were temperature of rectum, ear lobe, back, and abdomen as well as respiration and heart rates and Circulating levels of Prolactin and Cortisol hormones; which were measured for each doe at the beginning of the experiment (initial-Wk0) and at the end of two subsequent weeks (Wk1 and Wk2). The results showed that there were significant week differences between in Ear, back, abdomen temperatures as well as heart rate and Cortisol serum level. Treatment within week effect was significant in case of abdomen temperature and serum levels of Prolactin and Cortisol. However the effect of Treatment within week on respiratory rate was on border ($P \leq 0.07$). Circulating levels of Prolactin and Cortisol hormone as well as respiratory rate differed significantly ($P < 0.05$) using Duncan's Multiple Range Test, with a reduction trend in treated rabbit does compared to control ones. It can be concluded that treated rabbits with mint leaves extract might have an amelioration effect of high environmental temperature.

Keywords: Rabbits, Mint Leaves, Prolactin, Cortisol, High Temperature

INTRODUCTION

Summer heat stress adversely affects productive and reproductive functions in many mammalian species especially farm animals. Rabbits in particular are very sensitive to heat stress, probably due to lack of functional sweat glands and insufficient capability to get rid of excess heat during high ambient temperature (Marai *et al.*, 2002).

Recent reports indicated that mammalian cell experience formation of lipid peroxidation or reactive oxygen species after exposing to heat stress (Flanagan *et al.*, 1998; Lord Fontaine and Averill-Bates, 2002). Accumulation of these radical groups in serum and tissues to level exceeds their antioxidant capacity may cause cellular damage and dysfunctions (Mates *et al.*, 1999; Ozturk and Gumuslu, 2004). Therefore, many attempts have been made to enhance antioxidative enzymatic system to protect tissues from superoxide radicals. Mint (*Mentha longifolia*) leaves are among medicinal plants and herbs which have been used to inhibit oxidative stress and enhance cell survival during heat stress and thus productivity (Shobana and Naidu, 2000; Brown, 1995; Maini *et al.*, 2007). The aim of the current study was to evaluate the effect of gulping mint extract leaves on physiological and hormonal performances in heat sensitive rabbit does during summer.

MATERIALS AND METHODS

This study was carried out at the Experimental Farm of Faculty of Agriculture, Suez Canal University, Ismailia, Egypt, during summer season. Data were obtained from a total of 25 New Zealand White rabbit does, divided randomly into two groups. The first group was 10 multiparous does and served as a control group. While the second group was 15 multiparous does and served as a treatment group. The weight of does ranged from 2,500-3,500 kg and the average of body weight

was 3,021 kg. Does were reared in metal and galvanized individual cages in a semi closed rabbitry with a 16 hrs lighting period. The rabbits were fed on a commercial pelleted feed containing approximately 16.1% protein, 2.39% crude fat and 12.8% crude fiber. Feed and water were offered *ad libitum* all over the experimental period.

Preparation of mint leaves extraction was carried out by boiling water and then adding 3g of mint leaves in 200 ml boiled water for 10 minutes. The beaker was covered during boiling until used. Boiled water with mint leaves were allowed to cool to ambient temperature before use. A syringe of 10 ml was used to gulping an amount of 8 ml boiled mint leaves extraction through the mouth of each treated doe daily for 14 days. The dry bulb temperature and the relative humidity percentage recorded during experimental period and temperature humidity index (THI) was calculated according to Marai *et al.* (2001) using the following formula: $THI = db^{\circ}C - [(0.31 - 0.31 RH)(db^{\circ}C - 14.4)]$, where $db^{\circ}C$ = dry bulb temperature in Celsius and RH = relative humidity percentage/100. The values of (THI) for rabbits ranged from 29.83 to 30.66 at the first week, while ranged from 29.71 to 30.29 at the second week and the average was 30.28. According to Marai *et al.* (2001), the rabbits in the present study with average THI equals 30.28. were under very severe heat stress.

Table (1): The average of the temperature humidity index (THI) during the experimental period of Study.

The period	(THI)
The first week	30.17
The second week	30.39

During experimental period each doe was subjected to determination of the following physiological parameters: rectum, ear lobe, back, abdominal temperatures, heart and respiration rate. Rectal

temperature was measured by using medical thermometer while ear lobe, dorsal, and abdominal temperature were measured by using a fever scan (it is a test for temperature determination of external part of the body using color changes in fever scan tap). Respiration and pulse rates were measured for each doe by counting flank movements for one minute and by counting heart pulses for one minute, respectively. The physiological parameters were measured three times, at the beginning of the experiment (Wo) and at the subsequent two weeks of treatment (W1 and W2).

Blood samples were withdrawn and collected at the initial (Wk0), first (Wk1), and second (Wk2) weeks from each rabbit doe via median ear vein. Blood serum was obtained by allowing blood samples to clot overnight at 5° C. Thereafter, blood samples were centrifuged at 3000g for 20 minutes to collect blood serum. Blood serum samples were stored at -20° until prolactin and cortisol hormonal assay. Prolactin and cortisol hormones were assayed using direct immunoenzymatic assay (ELIZA technique). The kit was purchased from Dia Metra, S.I., Headquarter: Via Garibaldi, Italy.

Analysis of covariance was applied using nested design with the Treatments within weeks as fixed effect and the initial weight of does (at the start of the experiment) as a covariate using the procedure GLM of SAS (SAS, 1986). The analysis of covariance as well as Separation of means between treatments (within each week and for the whole period) was performed using Duncan's Multiple Range Test; according to procedures described by Steel and Torrie (1980).

RESULTS AND DISCUSSION

After two weeks of treating rabbit does with mint leaves extract, significant week differences between in Ear, back, abdomen temperatures ($P \leq 0.05$ -table not included) of drinking mint compared to control rabbits. Treatment within week effect was significant in case of abdomen temperature, However the effect of Treatment within week on respiratory rate was on border ($P \leq 0.07$).

The analysis of covariance (the mean separation presented in table 2) showed that respiratory rate differed significantly ($P \leq 0.05$) using Duncan's Multiple Range Test, with a reduction trend in treated rabbit does compared to control ones. This kind of response may alleviate of hot climate stress drawbacks on rabbits during summer season in Egypt. The current result may indicate improved heat tolerance in rabbit does treated with mint leaves extract. Although the mechanism by which mint exerts its effect is not very clear, but Mint is expected, as a medicinal plant, to include some constituent that have beneficial effect on rabbit tolerance to summer hot stress.

Some research workers observed that rabbits exposed to high environmental temperature suffered increased respiration rate (Kasa and Thwaites, 1992, Habeeb *et al.*, 1997), elevated ear lobe temperature (Shafie *et al.*, 1970) and rectal temperature (Habeeb *et al.*, 1998). Also, Johnson *et al.* (1957) reported that respiration rate increased rapidly from 69 to 190 breaths/min in NZW rabbits when environmental temperature increased from 10 to 30.8° C. The ear lobe temperature differs greatly during the different months of the year, correspondingly to air temperature changes (Shafie *et al.*, 1970).

Concerning the circulating Cortisol and Prolactin, there was a significant week effect on Cortisol serum level. However, treatment within week effect was significant in case of serum levels of both Prolactin and Cortisol.

Circulating levels of Prolactin and Cortisol hormone differed significantly ($P < 0.05$) using Duncan's Multiple Range Test in the first and the second weeks, with a general reduction trend in treated rabbit does compared to control. Stressors can induce Prolactin release from the anterior lobe of the pituitary gland (Colthorpe *et al.*, 1998; Bodnar *et al.*, 2004). Elevation level of prolactin as a response to stress can inhibit reproduction and cause anestrus (Kernabon *et al.*, 1994). The current finding that mint leaves extract attenuated Prolactin level may suggest positive effect on the function of the hypothalamus-pituitary-ovary axis.

Table (2): Actual means (\pm SE) of temperature of rectum, ear lobe and back, rate of heart beat and respiration in rabbit does on alleviation of summer hot stress (Mean \pm SE).

Parameters	Control		Mint	
	The first week	Second week	The first week	Second week
Rectum temperature	40.01 \pm 0.13	39.75 \pm 0.13	40.07 \pm 0.12	39.96 \pm 0.13
Average	39.88 \pm 0.09		40.01 \pm 0.09	
Ear temperature	37.40 \pm 0.41	38.45 \pm 0.27	37.37 \pm 0.37	38.23 \pm 0.34
Average	37.93 \pm 0.27		37.80 \pm 0.26	
Back temperature	36.80 \pm 0.30	37.60 \pm 0.15	36.63 \pm 0.24	37.30 \pm 0.19
Average	37.20 \pm 0.19		36.97 \pm 0.16	
Abdomen temperature	36.30 \pm 0.	37.25 \pm 0.20	36.57 \pm 0.16	36.97 \pm 0.14
Average	36.78 \pm 0.17		36.77 \pm 0.11	
Heart rate	116.20 \pm 2.43	120.00 \pm 3.10	114.40 \pm 2.58	121.20 \pm 2.21
Average	118.10 \pm 1.97		117.80 \pm 1.78	
Respiration rate	135.10 \pm 2.9 ^a	135.80 \pm 1.41 ^a	121.33 \pm 0.89 ^b	124.20 \pm 0.88 ^b
Average	135.45 \pm 1.27 ^a		122.77 \pm 0.67 ^b	

Means within each row with different superscripts differ significantly ($P < 0.05$) using Duncan's Multiple range test.

Table (2): Actual means (\pm SE) of levels of the circulating Cortisol and Prolactin hormones as affected by gulping rabbit does with mint leaves extract on alleviation of summer hot stress.

Parameters	control		mint	
	The first week	Second week	The first week	Second week
Cortisol hormone level	5.13 \pm 0.15 ^a	4.01 \pm 0.29 ^a	3.25 \pm 0.09 ^b	3.41 \pm 0.18 ^b
Average	4.57 \pm 0.20 ^a		3.33 \pm 0.10 ^b	
Prolactin hormone level	3.84 \pm 0.06 ^a	4.03 \pm 0.10 ^a	3.37 \pm 0.07 ^b	3.01 \pm 0.11 ^b
Average	3.94 \pm 0.06 ^a		3.19 \pm 0.07 ^b	

Means within each row with different superscripts differ significantly ($P < 0.05$) using Duncan's Multiple range test.

As for Cortisol picture, Christison and Johnson, (1972) reported that activation of the hypothalamic-pituitary-adrenal axis and the consequent increase of plasma glucocorticoid concentrations are perhaps the most important responses of animals to stressful conditions. Adrenal corticoids, mainly cortisol, elicit physiological adjustments which enable animals to tolerate stressful conditions. Decreased levels of Cortisol, though of insignificance which might be due to small sample size, may indicate an enhancement in does physiological status causing the hypothalamic-pituitary-adrenal axis to return to its normal status.

CONCLUSION

This present study showed that gulping of mint leaves extraction can partially alleviate hot summer stress in rabbit does under the surveillance conditions of Ismailia Provence.

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تأثير مستخلص أوراق النعناع علي بعض القياسات الفسيولوجية والهرمونية في الأرانب النيوزيلندية البيضاء خلال فترة الصيف

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استخدم في هذه الدراسة ٢٥ من الأرانب النيوزيلندي الأبيض والتي وزعت عشوائيا علي اثنين من المعاملات. المعاملة الأولى (١٠ أرانب) وتم استخدامها كمجموعة مقارنة بينما تضمنت المعاملة الثانية علي ١٥ أرنب كانت تتجرع مستخلص أوراق النعناع (٣ جم مسحوق جاف / ٢٠٠ مل ماء مغلي) بشكل يومي ولمدة ١٤ يوم. والصفات التي تم تقييمها لدراسة تأثير المعاملة كانت قياس درجة حرارة المستقيم وفص الأذن والظهر والبطن ومعدل التنفس وقياس معدل ضربات القلب لكل أرنب كذلك تم تقدير مستويات هرموني البرولاكتين و الكورتيزول لكل أرنب عند بداية التجربة وفي نهاية كل من الأسبوعين اللاحقين.

أظهرت النتائج وجود فروق معنوية لتأثير الأسبوع علي درجات حرارة الأذن والظهر والبطن ومعدل ضربات القلب ومستوى هرمون الكورتيزول. وكان تأثير المعاملة داخل الأسبوع معنويا في حالة درجة حرارة البطن ومستوى هرمونات البرولاكتين والكورتيزول وأعطى تأثير المعاملة معنوية علي معدل التنفس قرب المستويات المعهودة عند ($P \leq 0.07$).

أظهر إحتبار دانكن للفرق بين المتوسطات تأثيرا معنويا علي معدل التنفس ومستويات هرمونات البرولاكتين والكورتيزول مع وجود اتجاه لإنخفاضها في الارانب المعاملة بمستخلص النعناع مقارنة بمجموعة المقارنة. أظهرت النتائج أن معاملة الأرانب بمستخلص أوراق النعناع قد يكون له تأثير مقلل لأثر درجات الحرارة العالية في فصل الصيف علي إناث الأرانب البالغة.