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Increased Antioxidant and White Blood Cell Counts and Decreased Free Radical Production During Mild Heat Stress in *Uromastyx egyptius*

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The primary purpose of this study was to evaluate the effect of mild heat stress on the activity of Biological Antioxidant Potential (BAP), Free Radicals production (FR) and white blood cell count (WBC) in spiny tailed lizard, *Uromastyx egyptius*. The experimental group underwent mild-moderate heat stress (33-35°C) for six weeks period. In comparison with the control group FR production decreased significantly. The biological antioxidant potential, WBC and lymphocyte were significantly higher in the experimental group. The regression of BAP on WBC was significant in the experimental group. There were no significant changes in the enzymes of liver function profile in terms of AST, ALT and LDH activities. These results indicate that mild heat stress induces positive physiological responses that related to the activities of antioxidant enzymes and immune cells proliferation and differentiation.

Key words: Antioxidant, free radicals, mild heat stress, white blood cells

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INTRODUCTION

Maintenance of healthy living environmental conditions have been a vital aspect of environmental medicine. Environmental temperature is essential to increase the body's abilities to resist tissue ischemia and related cellular damages as well as free radical production. Heat stress has been studied since the middle of the 19th century in attempt to understand the physiological and biochemical basis of mechanisms underlying the cellular response. Hyperthermia is known to induce free radical production. Reactive Oxygen Species (ROS) such as super oxide ($O_2^{\bullet}$) are produced during hyperthermia[1-5].

While most investigators have focused on the pathophysiological effects of hyperthermia, the cellular response to moderate or mild heat stress has been ignored. The cellular response to mild heat stress is more likely to have more important physiological and medical applications, especially in intensive care units. It has been widely accepted that the increase in thermal tolerance play an essential role in protection against cellular damage. It is likely that the mild to moderate heat stimulus stimulate endogenous antioxidant defenses. It is not clear how heat stimulus moderate white blood cells proliferation and differentiation.

Lizards are ectotherm that requires specific high temperature range as compared to mammals mainly due to their physiological adaptation against dehydration[6-10]. It is not known how Mild Heat Stress (MHS) stimulates the antioxidant system to resists ROS generation. The relationship between white blood cells proliferation and BAP has not been examined. Here we provide medical information with regard to the physiological benefits of MHS and the role that has on stimulating the antioxidant system, white blood cells and biochemical hepatocytes responses to mild heat stress in *Uromastyx egypitus*. In the present study we mimic the lizard's native temperature in order to examine Free Radical (FR) production, Biological Antioxidant Potential (BAP) and white blood cell (WBC) as well as liver function profile.

MATERIALS AND METHODS

Animals and experimental design: All animals were obtained from the central region in the Kingdom of Saudi Arabia and kept 3 days to adjust to laboratory condition. Nineteen adult lizards, *Uromastyx egyptius,* were divided randomly into two groups, nine experimental and ten controls. The experimental group underwent whole body mild-moderate heat exposure (33-35°C) for six week period. This temperature range was considered to induce mild heat stress in lizards. The control group was kept at the laboratory room temperature (22-24°C). All animals were fed mixed greens obtained from their native environment and all were kept under the same light conditions, 12 h light and 12 h dark.

Heat stress calculations: Heat stress calculations were conducted as described by Haffor and Al-Johany[5].

Statistical analysis: Mean group differences for free radicals, biological antioxidant and white blood cell percent were evaluated using t-test. A multiple regression model for BAP versus WBC relationship was generated using least square linear regression model. Residual sum of squares criterion was used to evaluate the validity of the regression model.

Blood sample collection: Three to five milliliter of blood samples were collected into heparinized syringe by heart puncture technique. Ten blood samples from the control and nine samples from the experimental group were used for FR and BAP measurements by D-ROM method according to Fenton reaction as described by Haffor[11]. Eight blood samples from the two groups were used for blood cells count, using hematology system (ADVIA, Bayer Corp., N.Y. and USA). In order to assess liver function additional seven blood samples from the two groups were used for lactate dehydrogenase (LDH), aspartate amino transferase (AST) and alanine aminotransferase (ALT), Albumin (ALB), cholesterol (CHO), triglycerides (TG) and total protein (TP).

RESULTS

Results showed that FR production was lower significantly ($p<0.05$) by 29% in experimental group, as compared with the control group. On the contrary the BAP was lower ($p<0.05$) in the control group (Table 1).

Table 1: FR and BAP for control exposed to 22-24°C and experimental groups exposed to 32:34°C

Control (22-24°C)		Experimental (32-34°C)	
Free radicals (CARR U)	BAP (μL)	Free radicals (CARR U)	BAP (μL)
81	4594.00	77.00	4630.00
130	3254.00	128.00	3952.00
171	3505.00	91.00	4294.00
129	3659.00	80.00	4774.00
156	4426.00	56.00	5137.00
140	4275.00	93.00	4585.00
136	4732.00	128.00	5293.00
122	4084.00	92.00	5113.00
133	4426.00	84.00	4050.00
115	----		---
131.3	4106.11	92.11	4647.56
7.58	172.84	6.90	160.68

*Data were average of triplicate measurements based on 9 animals for BAP

There was no significant difference between the two groups in liver function profile in terms of the activity of the enzymes induced by cellular damage. The mean activity of AST, ALT and LDH were 50.43±8.83, 23.86±8.56 and 54.03±31.92 U L^{-1} in the control group (Table 2). The corresponding mean values in the experimental group were 36.57±11.87, 13.29±1.01 and 133±64.00 U L^{-1} (Table 3). Furthermore there were no changes in the biochemical variable as it relates to TP, ALB, CHO and TRIG in the control group (Table 2) and in the experimental group (Table 3). The mean white blood cell count and the percentage of lymphocyte were 167±10.65 and 81.25±4.87 in the control group (Table 4). The corresponding values in the experimental group were 208.15±9.18 and 91.75±1.53 (Table 5) which were significantly ($p<0.05$) higher.

Furthermore regression analysis indicated a significant ($p<0.05$) relationship between leukocytes and BAP ($R^2 = 0.67$) in the experimental group (Fig. 1). This regression model was evaluated using residual sum of squares which departed symmetrically from zero at all levels of WBC (Fig. 2).

Table 2: Liver function profile in the control group

Animal	AST (U L^{-1})	ALT (U L^{-1})	AST/ALT (Ratio)	ALB (g L^{-1})	TP (g L^{-1})	LDH (U L^{-1})	CHOL (mmol L^{-1})	TG (mmol L^{-1})
1	34.00	13.00	2.62	7.00	43.00	41.00	4.90	0.40
2	87.00	16.00	5.44	8.00	48.00	15.00	6.00	3.50
3	39.00	14.00	2.79	9.00	47.00	46.00	5.10	6.30
4	21.00	9.00	2.33	5.00	32.00	3.40	3.40	3.40
5	70.00	25.00	2.80	4.00	34.00	6.78	3.30	11.40
6	39.00	16.00	2.44	4.00	26.00	24.00	2.20	0.10
7	63.00	74.00	0.85	7.00	38.00	242.00	3.40	14.60
Average	50.43	23.86	2.75	6.29	38.29	54.03	4.04	5.67
SEM	8.83	8.56	0.51	0.75	3.09	31.92	0.50	2.07

Table 3: Liver function profile in the experimental group

Measure/Animal	AST (U L^{-1})	ALT (U L^{-1})	AST/ALT (Ratio)	ALB (g L^{-1})	TP (g L^{-1})	LDH (U L^{-1})	CHOL (mmol L^{-1})	TG (mmol L^{-1})
1	28.00	18.00	1.56	8.00	39.00	21.00	8.70	0.50
2	25.00	11.00	2.27	7.00	39.00	82.00	8.90	7.50
3	78.00	13.00	6.00	6.00	34.00	443.00	8.00	1.20
4	10.00	12.00	0.83	8.00	36.00	25.00	11.60	0.20
5	12.00	11.00	1.09	6.00	36.00	33.00	9.70	5.30
6	85.00	16.00	5.31	6.00	30.00	300.00	4.50	0.30
7	18.00	12.00	1.50	6.00	37.00	27.00	28.00	2.10
Average	36.57	13.29	2.65	6.71	35.86	133.00	11.34	2.44
SEM	11.87	1.01	0.79	0.36	1.18	64.00	2.89	1.78

Table 4: Hematology profile in the control group

Measure/Animal	WBC (10^3 mm^{-1})	RBC (10^6 mm^{-1})	Hb (g dL^{-1})	HCT (%)	Neut (%)	Lymph (%)	Mono (%)
1	180.00	0.02	8.40	0.40	7.00	90.00	1.00
2	187.10	0.01	8.40	0.30	10.00	86.00	1.00
3	140.60	0.02	6.80	0.40	11.00	89.00	0.00
4	127.30	0.02	5.50	0.30	26.00	50.00	2.00
5	190.00	0.01	2.10	0.20	8.00	90.00	2.00
6	196.20	0.02	8.40	0.30	16.00	80.00	1.00
7	127.10	0.02	5.50	0.30	25.00	75.00	0.00
8	190.00	0.01	2.10	0.20	8.00	90.00	2.00
Average	167.29	0.02	5.90	0.30	13.88	81.25	1.13
SEM	10.65	0.0018	0.93	0.027	2.72	4.87	0.30

Table 5: Hematology profile in the experimental group

Measure/Animal	WBC (10^3 mm^{-1})	RBC (10^6 mm^{-1})	Hb (g dL^{-1})	HCT (%)	Neut (%)	Lymph (%)	Mono (%)
1	226.00	0.03	9.80	0.40	10.00	87.00	3.00
2	149.80	0.01	6.70	0.40	3.00	96.00	1.00
3	209.00	0.02	8.90	0.30	2.00	95.00	2.00
4	208.90	0.03	8.70	0.50	10.00	88.00	2.00
5	219.50	0.02	8.60	0.30	3.00	94.00	3.00
6	200.00	0.01	2.10	0.20	10.00	85.00	5.00
7	235.00	0.02	11.10	0.40	3.00	94.00	1.00
8	217.00	0.02	8.90	0.50	5.00	95.00	1.00
Average	208.15	0.02	8.10	0.38	5.75	91.75	2.25
SEM	9.18	0.00267	0.96	0.037	1.28	1.53	0.49

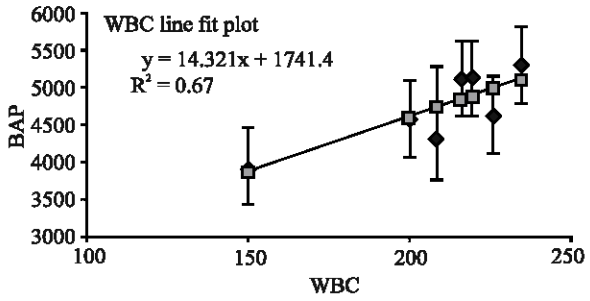

Fig. 1: The regression of BAP on WBC in the experimental group

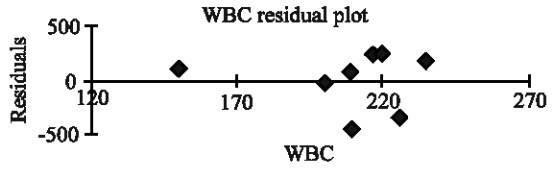

Fig. 2: Residual plot of the regression of BAP on WBC in the experimental group

DISCUSSION

The results of the present study indicated that mild heat stress resulted in increased activities of BAP and decreased FR productions in *Uromastyx egyptius*. Previous work from our laboratory showed elevated free radicals production induced by high level of heat stress in mice which was similar to free radicals production induced by hypoxia and hpoxia-hyperoxia[5]. Therefore high levels of heat stress and ROS production are closely related in terms of increased rate of FR formation that promote cellular damages. It was shown that there was an increase in ROS production during 42°C exposure[12,13]. In addition it was also reported that hyperthermia leads to oxidants production that was related to heat shock protein-HSP[14] and increased frequency of apoptosis-like cell as indicators of programmed cell death[4] and nuclear mutation[15]. As the origin of oxidative stress and or reductive stress is the mitochondrial respiratory electron transport chains[12,16-19], it can be suggested that the cellular damages induced by heat stress loop is mediated by mitochondrial stimulus.

The findings of lower free radicals production and the absence of liver cells damages in the present study suggest that molecular feedback loop is modified under different heat conditions. In other words, it can be suggested that mild heat stress act as cellular stimulus to activate antioxidant enzymes or stimulate immune response or both. In the mean time, the possibility of synthesizing HSP formation can be excluded in mild heat stress because its stimulus is transient and requires high level of heat induction. Thus mild heat stress have some positive role in organisms via positively regulating cell proliferation and differentiation similar to those observed in WBC changes of the present study. Several clinical studies[20-24] reported that moderate heat stress play an essential role in delayed protection by ischemic preconditioning and improved cardio thrombi protection against ischemia/reperfusion injury[25-28]. Moreover, some studies reported that heat shock preconditioning 24 h before an ischemic event leads to an up-regulation of heat protein 72 in muscle and to a tissue protection reducing ischemia-reperfusion injury in composite tissue transplantation[10,23,29].

Based on the results of the present study it can be concluded that MHS stimulate antioxidant defense system, immune response and lower free radical production and subsequently act as protection against cellular damages.

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