IMPAIRED EPIDIDYMAL FUNCTION AND CHANGES IN THYROID AND ADRENAL GLANDS MORPHOLOGY OF HEAT STRESSED RATS

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Received *September 25, 2019;* ***Revised*** *November 20, 2019;* ***Accepted*** *November 28, 2019*

ABSTRACT

Twenty mature male albino rats randomly assigned to two groups were used for the study. In group A rats, hyperthermia was induced exogenously by exposure to the sun, while group B rats were kept under a shade. The duration of the study was 28 days. At the end of the study the effects of heat stress on body weight (BW), body mass index (BMI), feed intake (FI), epididymal sperm reserve (ESR), thyroid and adrenal glands morphology were assessed. On day 28, rats that were exposed to heat stress showed significant (p<0.05) decreases in their mean BW and FI when compared to the unexposed. The BMI of the heat stress exposed rats and unexposed rats remained unchanged throughout the period of study. Epididymal sperm reserve was significantly reduced (p<0.05) in the heat stressed rats when compared to the unexposed rats. Histological examination of the thyroid and adrenal glands of the heat stressed rats revealed hypertrophy and hyperplasia of the thyroid and adrenal glands respectively when compared to unexposed rats.

Keywords: Heat stress rat, Body weight, Body mass index, Epididymis, Thyroid gland, Adrenal gland

INTRODUCTION

Heat stress (HS) causes hyperthermia in rats. The resulting hyperthermia has negative effects on feed intake and daily weight gain and also causes lipid peroxidation in the liver of rats (Ando *et al*., 1997). Lipid peroxidation in the liver of rats exposed to heat stress contributes to the overall deleterious effects on their physiological status (Ando *et al*., 1997) due to increased levels of oxidative stress (Dahl *et al*., 2012). Heat stress reduces feed intake in rat in response to reduce the body heat (Sengupta and Sharma, 1993). Rats under HS conditions increase both respiratory rate and water intake, and reduce feed intake and serum T3 resulting in reduced metabolic heat production levels to maintain homeothermy (Bozkawa *et al*., 2016). In conditions where these avenues of heat loss and production fail to maintain homeothermy, animals experience hyperthermia. Heat stress causes protein structure instability throughout the body leading to deleterious denaturation of various essential proteins (Dahl *et al.,* 2012). Studies have shown that female rats mated by male rats exposed to heat stress come back to heat (Khan and Brown, 2002). Under chronic stressful conditions, blood circulating thyroid hormone levels decreases resulting in lower metabolic rate (Bozkawa *et al*., 2016). Hyperthermia is also an important stress factor and known to increase blood cortisol levels (Pignatelli *et al*., 1996). Hence, the objective of this study was to determine the epididymal sperm reserve and morphological changes of

thyroid and adrenal glands of male rats exposed to heat stress.

MATERIALS AND METHODS

Animals: Twenty male rats of twelve weeks old, weighing between 180 and 200 g were used for the study. They were procured from the Animal House, Department of Veterinary Anatomy, University of Nigeria, Nsukka. The ethical committee of the university approved the study protocol prior to commencement of the study. The study was carried out in accordance with the guidelines for ethical conduct in the care and use of nonhuman animals in research (APA, 2012).The animals were kept in cages under 12 hours conditions of light, temperature (≃24 ^{o}C) and relative humidity (≃55 %), and fed *ad libitum* with pelleted grower's mash and water. The animals were acclimatized for two weeks before the commencement of the experiment.

Induction of Heat Stress in Rats: The animals were randomly assigned into two groups of 10 rats each. The animals in group A were housed in a cage and exposed to the sun while those in group B were housed in a cage and kept under a shade. The study lasted for 28 days.

Determination of Some HS Associated Parameters: Daily feed intake was assessed by measuring the quantity of feed served and subtracting the remnant of feed after 24 hours (Sengupta and Sharma, 1993). Body weights (g) of rats were measured using an electronic weighing balance. The body mass index was determined using a formula = weight in Kg/Height^2 in meters (Novelli *et al*., 2007). Epididymal sperm reserves were determined using the haemocytometer method (Obidike *et al.,* 2012). The histopathology of the thyroid and adrenal glands was done using the standard procedures described by Odo *et al.* (2016).

Statistical Analysis: Data obtained from the study were analyzed using independent T-test. Level of significance was accepted at $p<0.05$. The results were presented as means ± standard error in tables.

RESULTS

On day 14, rats that were exposed to heat stress showed significant ($p<0.05$) decreases in their mean BW (81.00 ± 3.18 g) and FI (89.27 ± 1.57 g) when compared to the unexposed rats (BW 106.10 ± 4.53 g, FI 103.06 ± 1.05 g) (Tables 1 and 2). On day 28, rats that were exposed to heat stress also showed significant decreases ($p<0.05$) in their mean BW (94.50 ± 3.07 g) and FI (110.11 ± 1.36 g) when compared to the unexposed rats (BW 124.20 ± 4.79 g, FI 127.51 ± 1.67 g) (Tables 1 and 2).

Table 1: Effect of heat stress on body weight of rats exposed for 28 days

Days	Body weight (g)	
	Group A (Heat exposed)	Group B (Heat unexposed)
0	66.20 ± 2.20	67.05 ± 3.21
14	81.00 ± 3.18^{a}	106.10 ± 4.53^{b}
28	94.50 ± 3.07^{a}	124.20 ± 4.79^{b}

a,b = means with different superscript are significantly different (p<0.05)

Table 2: Effect of heat stress on feed intake of rats exposed for 28 days

Days	Feed Intake (g)	
	Group A (Heat exposed)	Group B (Heat unexposed)
14	89.27 ± 1.57^{a}	103.06 ± 1.05^{b}
28	110.11 ± 1.36^{a}	127.51 ± 1.67^{b}

a,b = means with different superscript are significantly different (p<0.05)

The BMI of the heat exposed rats (day 14 - 22.87 ± 0.91, day 28 - 21.72 ± 0.83) and unexposed rats (day 14 - 22.95 ± 0.55, day 28 - 22.14 ± 0.44) remained unchanged throughout the period of study (Table 3). Epididymal sperm reserve was significantly reduced in the heat stressed group (45.52 ± 4.18) when compared to the unexposed group (139.36 ± 20.92) (Table 4). Histological examination of the thyroid and adrenal glands from heat stressed group revealed hypertrophy of the thyroid follicle (Figure 1) and hyperplasia of the adrenal

medulla (Figure 2) when compared to unexposed group showing normal morphology of the thyroid gland with normal sized follicles (Figure 3) and normal morphology of the adrenal gland with normal sized medulla (Figure 4).

Table 3: Effect of heat stress on body mass index of rats exposed for 28 days

Days	Body mass index	
	Group A (Heat exposed)	Group B (Heat unexposed)
14	22.87 ± 0.91	22.95 ± 0.55
28	21.72 ± 0.83	22.14 ± 0.44

a,b = means with different superscript are significantly different (p<0.05)

Table 4: Effect of heat stress on epididymal sperm reserve of rats exposed for 28 days

Parameter	Group A (Heat exposed)	Group B (Heat unexposed)
Epididymal sperm reserve ($x10^6$)	45.52 ± 4.18[a]	139.36 ± 20.92[b]

a,b = means with different superscript are significantly different (p<0.05)

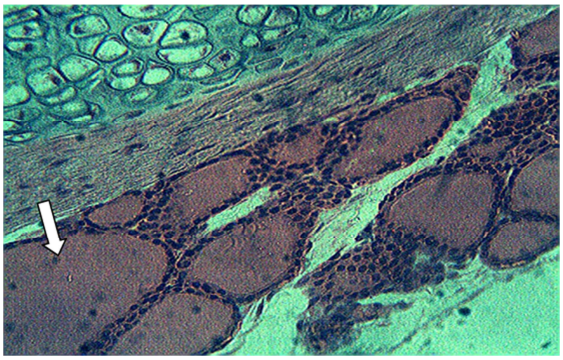

Figure 1: Thyroid gland from heat stressed rat showing hypertrophy of the thyroid follicle (arrow) as a result of prolong exposure to heat stress, H & E, Mag. x200

DISCUSSION

The significant decrease (p<0.05) in BW of heat stressed rats when compared with the unstressed group may be as a result of reduced feed intake. The significant decrease in feed intake in heat stressed rats when compared with unstressed may be an attempt to reduce the metabolic rate hence reducing the metabolic body heat that could have been generated in order to maintain homeostasis (Gordon, 2017). The result of the BMI showed no significant difference (p>0.05) between groups A and B. This suggests that the exposure to heat stress did not cause any effect on the BMI.

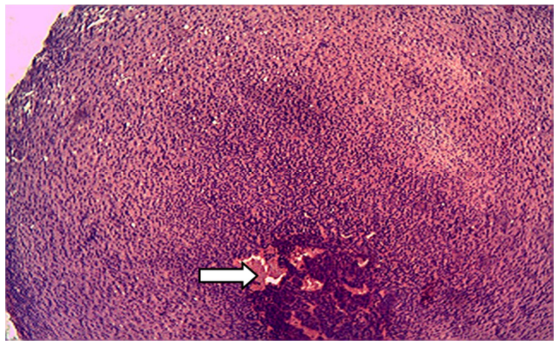

Figure 2: Adrenal gland from heat stressed rat showing hyperplasia of adrenal medulla (arrow) as a result of prolong exposure to heat stress, H & E, Mag. x200

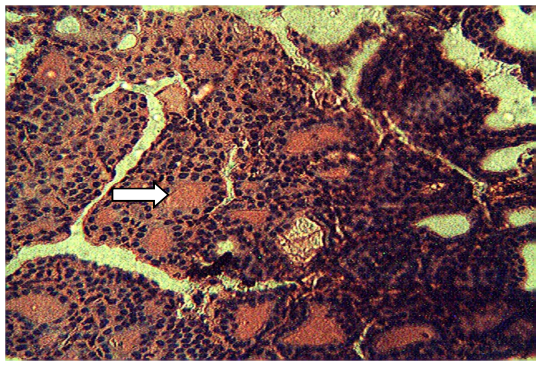

Figure 3: Thyroid from heat unstressed rat showing normal morphology of the thyroid gland with normal sized follicles (arrow), H & E, Mag. x200

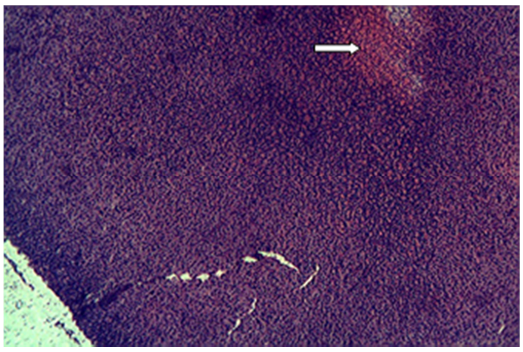

Figure 4: Adrenal gland from heat unstressed rat showing normal morphology of the adrenal gland with normal sized medulla (arrow), H & E, Mag. x200

This is supported by Novelli *et al.* (2007) that for a given height, body mass index (BMI) is proportional to the weight. However, for a given weight, BMI is inversely proportional to the square of the height. Heat stress thus has no influence on BMI i.e. BW divided by height^2 (Novelli *et al*., 2007).

The significant decrease in epididymal sperm reserve of the heat stressed group when compared with unstressed group may be as a result of heat stress effect on the gonadal hormone and all phases of semen production (Bozkawa *et al.,* 2016). Bozkawa *et al*. (2016) concluded that stress due to high environmental temperature disturbs the pulsatile-gonadotropin-releasing hormone generator frequency, which in turn impairs reproductive function due to heat impaired gonadal hormone.

The result of the histology of the thyroid gland showed a markedly distention of the follicular cells of the thyroid gland and reduction in colloid droplets in heat stressed group when compared with the control. This may be as a result of poor absorption of iodine to reduce the metabolic rate which could have led to generation of more heat to the body. Consequent decrease of iodine concentration in plasma may have led to compensatory enlargement of the follicles (Mete *et al*., 2012).

The result of the histology of the adrenal gland showed clumping or hyperplastic change of the medulla of the heat stressed group when compared with the normal. This may be due to an increase in catecholamines production as a result of heat stress. Stress is associated with increase in the production of catecholamines by the adrenal medulla (Milovanovic *et al*., 2003).

Conclusion: The results from this study revealed significant harmful effects of heat stress on physiological parameters assessed, hence shade should be provided in animal houses.

ACKNOWLEDGEMENT

We thank Mrs. Martina Onu of the Department of Veterinary Physiology and Pharmacology, University of Nigeria, Nsukka for her excellent technical assistance.

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