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Effects of Cold and Heat Stress on the Rat Adrenal Glands

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ABSTRACT
Present study was performed to examine the effect of heat on rat adrenal glands. After the weaning period (week 3), 54 Sprague Dawley rats were divided into three main groups, each consisting of twelve animals. For each group, 2 sub-groups were constituted (6 males and 6 females). After slaughtering, adrenal glands were rapidly removed and semi-quantitatively analyzed by conventional histology. The study took placed in the Department of Medical Science Application and Research Center of Ataturk University, Erzurum; Turkey. The rats were sacrificed by decapitation and the left adrenal gland of each animal was dissected out and prepared for morphometric analyses. This investigation describes histological and cytometrical changes of cortical and medullary tissue of adrenal in rats under the acute and chronic stress. Adrenal weight decreased for males and increased for females, respectively, with increasing sress duration. Although volume density of zona glomerulosa and medulla was significantly increased, capsula, zona glomerulosa and zona fasciculata increased, in the treatment groups. We determined that after both stress types and stress duration, the adrenal gland showed histological changes both in the cortical and medullary region.
Key words: *Adrenal glands, cold, cortex, heat, medulla, stress.*

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I. INTRODUCTION

HPA (hypothalamic-pituitary-adrenal) axis is activated in any type of stress (1, 2) but the adrenal gland response varies (3-5). ACTH is the major stimulator of adrenal cortical function; investigation of their relationship is of great importance in both basal and stress conditions (2). Short-term ACTH treatment provokes a decrease in volume of the lipid-droplet compartments in rat zona glomerulosa (ZG) cells, and a rise in plasma and intracellular concentrations of corticosterone and aldosterone (5). The trophic effect of ACTH involves an increase in adrenal mass and in the steroidogenic capacity of adrenocortical cells (3).

Literatures suggest that very few studies related with heat-induced pathophysiological changes are clinically justified. Further, the majority of the reports are involved in the study of pathophysiology either under hyperthermia or under acute heat stress (6, 7). Therefore, in the present paper an effort has been made to study the effects of cold and heat stress on the adrenal glands in the rat.

II. MATERIALS AND METHODS

Animal and protocol design:
The rats of Sprague Dawley strain, 60-90 days old, weighing 180-220 g, were used for the experiments. The rats were divided into three main groups, each consisting of twelve animals. For each group, 2 sub-groups were constituted (6 males and 6 females). In all cases, females and males were separately allotted. The first group represented intact controls.

Acute heat stress: Each rat of this group was subjected to a single exposure in the incubator at the temperature of 38 ± 1°C for two hours from 8.00 A.M. to 12.00 P.M.

Chronic heat stress: Rats were subjected to a chronic heat exposure for one hour daily for 14 days from 8.00 A.M. to 9.00 A.M. at 38 ± 1°C.

Acute cold stress: Rats were subjected to a single exposure in the incubator at the temperature of 4 ± 1°C for two hours from 8.00 A.M. to 12.00 P.M.

Chronic cold stress: Rats were subjected to a chronic cold exposure in the incubator for one hour daily for 14 days from 8.00 A.M. to 9.00 A.M. at 4 ± 1°C.

Control: Rats were handled and processed as the acute and chronic stressed rats, respectively, but at controlled incubator temperature of 24 ± 1°C (same as the room temperature). These groups of rats were treated as controls for acute and chronic heat stressed groups.

Animals were allowed to access to standard rat diet and water *ad libitum* throughout the experiment. They were maintained on a constant 12-hour light/dark cycle with a relative temperature of 22 ± 2°C and with a 60-80% relative humidity.
At the end of the experiment, rats were firstly sedated by intraperitoneal injection of xylazine hydrochloride (Rompun, Bayer, Istanbul, Turkey, 5 mg/kg) then anesthetized by 2% sevoflurane (Sevorane, Abbott Laboratories, Istanbul, Turkey) and euthanized by exsanguinations. Six rats chosen randomly from each group were then euthanized by exsanguinations under anesthesia.

***Histological analysis*:**

Adrenal glands were removed and immersed in neutral buffered formaldehyde. After embedding the tissues in paraffin, complete serial sections (5 µm thickness) were cut and stained with hematoxylin eosin. Photomicrographs were taken with a camera attached to a microscope. Micrometric measurements and volume were carried out with ocular micrometer.

The adrenal glands were rapidly removed from the fresh cadaver, subsequently cut open and washed with running water and immediately immersed in 10% formalin. The segments were then embedded in paraffin blocks. Following this, sections of 5 µm in thickness were cut and stained with haematoxylin eosin. The stained sections were observed under light microscope (8). All results were tabulated for subsequent statistical study.

III. RESULTS

Macroscopically there were no differences between the adrenal glands of the treatment group and the control group. They were located on the upper poles of the kidneys at their normal position. The adrenal glands have 2 triangular, flattened glands that lie embedded in fatty tissue overlying the kidneys.
Histological investigation of adrenal gland revealed the existence of dark and light regions in the adrenal cortex of chronic stressed rats (Figure 1). The cells in the light regions were filled with large lipid droplets but those in the dark cortical regions were deprived of them.

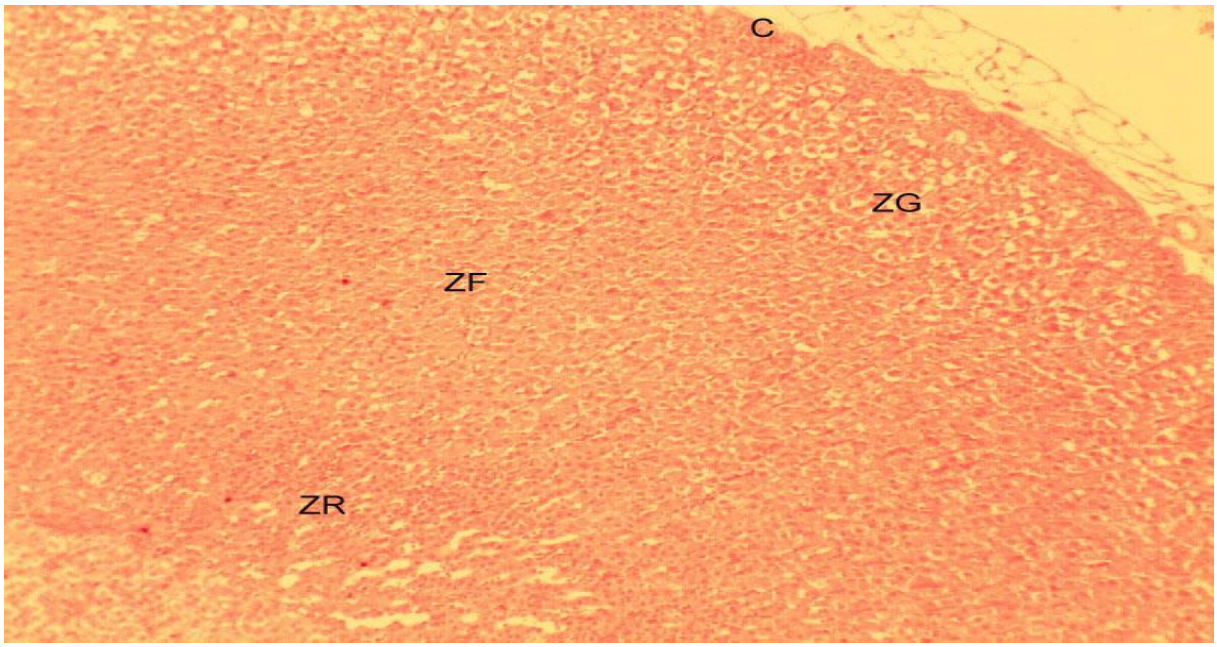

Figure 1. Light micrograph of dark and light regions in the adrenal cortex of control rats. C =capsula, ZG=zona glomerulosa, ZF=zona fasiculata, ZR=zona reticularis, CX=cortex, H&E stain, scale bars, 100 µm.

Total dimensions and thickness of cortex and its zones and thickness of medulla in adrenal glands were evaluated according to morphometric data with micrometric ocular in the treatment and the control groups (Table 1).
The effects of sress type, stress duration, and sex on the adrenal glands components and their ratio (%) was shown table 2.
The rat adrenal glands were bound externally by a thin fibrous capsule containing adipose tissue. Collagenous fibres and vessels were entered to inside of adrenal gland from the capsule. The adrenal cortex was comprised about 60-70 % of the gland and surrounds the centrally located medulla (Table 2).

Chronic stress induced a significant reduction of absolute and relative adrenal mass due to the reduction of cortical mass, especially that of zona fasiculata (ZF). This was expected because glucocorticoid synthesis is performed mostly in the ZF and this is the largest part of the cortex.

Cells in the adrenal cortex were arranged into three concentric zones. The outermost zone was the zona glomerulosa (ZG). The zona glomerulosa was about 7-9 % of the cortex under the capsule and contained foci of cells. Cells within this zone tend to be columnar in shape and were arranged in irregular cords. The whole ZG area was reduced and filled with enlarged cells and nuclei. Condensed nuclei were observed in zona reticularis (ZR) cells. There were no mitoses visible in the adrenal glands of rats in all groups. In cold stressed rats, however, all components of the adrenal glands were clearly differentiated (Figure 2). ZG of control rats contained many nuclei, as compared to ZG cells in stressed rats, and many mitotic figures were present in the outer cortical zones (ZF in close proximity to ZG).

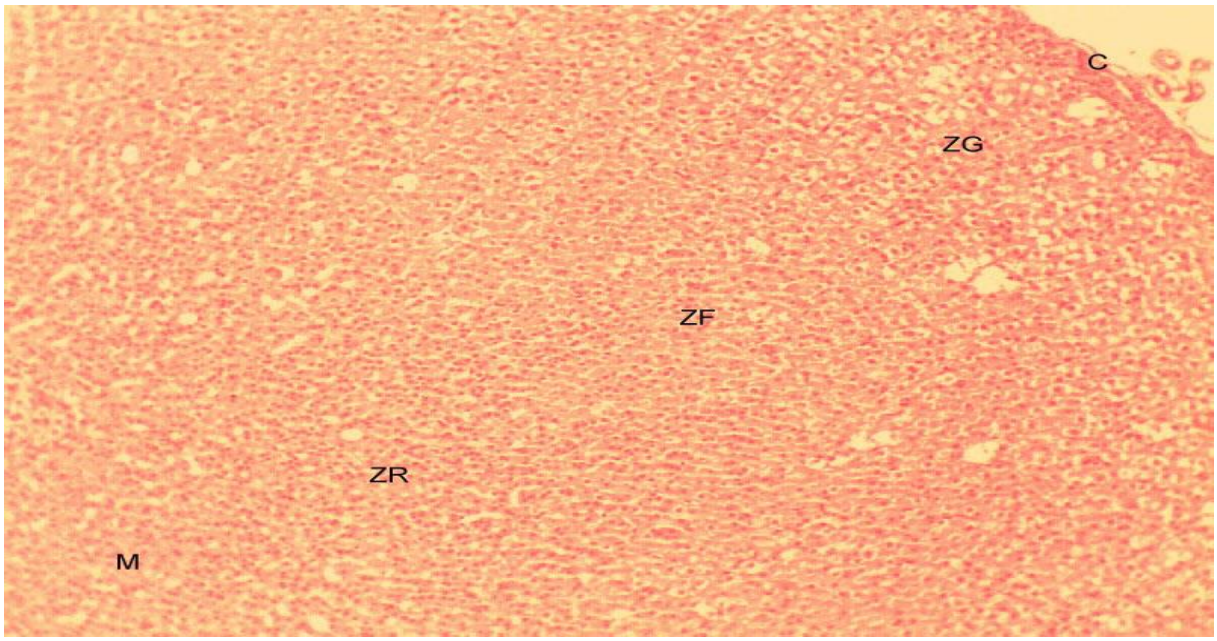

Figure 2. Light micrograph of all components of adrenal gland of cold stressed rats.
C =capsula, ZG=zona glomerulosa, ZF=zona fasiculata, ZR=zona reticularis, M=medulla, CX=cortex, H&E stain, scale bars, 100 µm.

Zona fasiculata, the middle zone of the adrenal cortex, cells in this zone were arranged in long cords or fascicles, which were separated by sinusoidal boundaries. It was the middle and largest of the three zones in the cortex. Cells of zona fasciculata, about 70% of the cortex, appeared to be vacuolated or clear on stained sections because of their high cholesterol content. Cells in the fasiculata were polyhedral and have a foamy appearance. They also were arranged in distinctively straight cords that radiate toward the medulla (Figure 3).

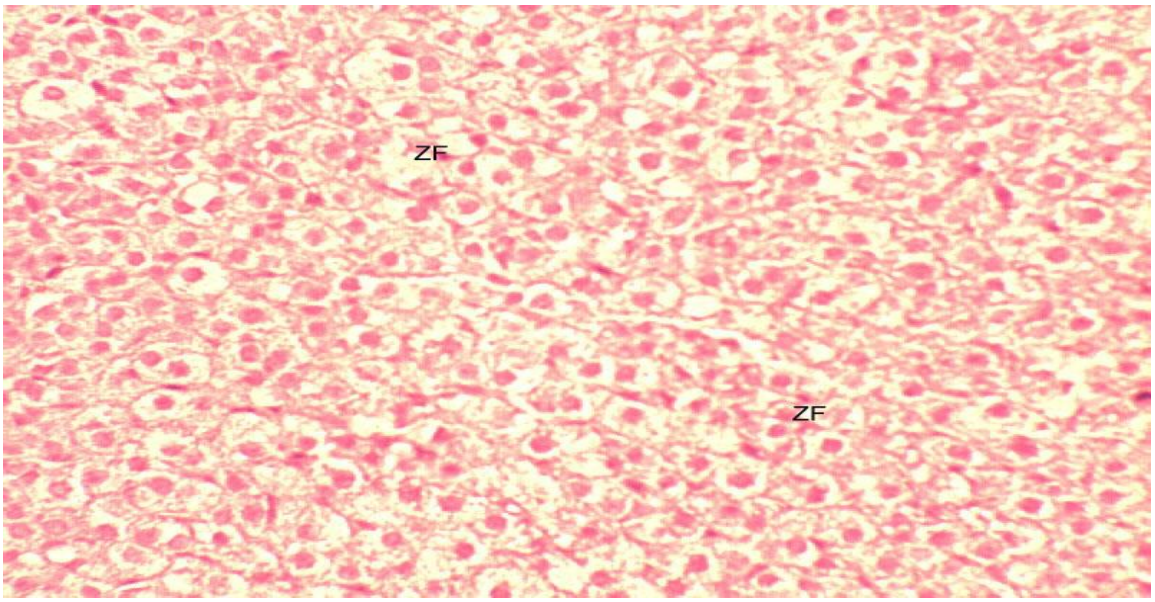

Figure 3. Light micrograph of zona fasiculata of adrenal cortex of heat stressed rats. ZF= zona fasiculata zona fasiculata, H&E stain, scale bars, 400 µm.

Zona reticularis, the inner zone of the adrenal cortex, cells in this zone were smaller than those in the zona fasiculata, and were arranged in an irregular network. All the nuclei in ZR of at acute cold stressed rats were large and light. Zona reticularis, about 20% of the cortex contained more compact cells with fewer lipids.

In the control group, zona reticularis cells were arranged in a spongelike meshwork of gently buckled anastomosing one-cell wide rows of cells that were separated by dilated capillaries. The well-outlined cells were smaller than those of the zona fasciculata and these cells had cytoplasm that is granular, acidophilic, and relatively lipid sparse (Figure 4).

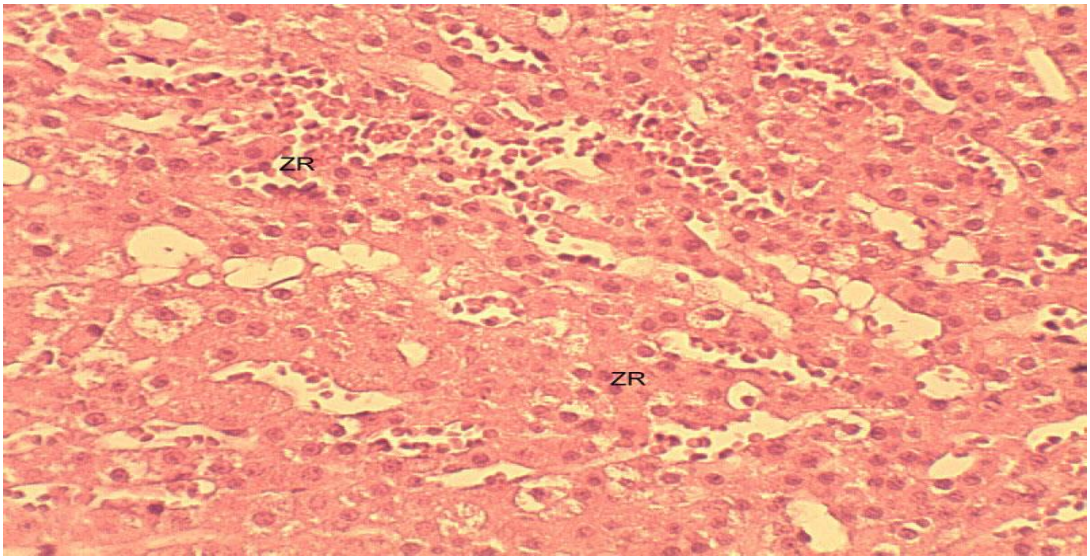

Figure 4. Light micrograph of zona reticularis of adrenal cortex of cold stressed rats. ZR= zona reticularis zona reticularis, H&E stain, scale bars, 400 µm.

It has been observed that both in the cortical and medullary region of the adrenal glands were affected from both cold stress and heat stress. Although there was a slight increase thickness of capsula, zona glomerulosa and zona fasciculate, there was a decrease at the zona glomerulosa and medulla (Tablo 1).

The most abundant cell in the adrenal medulla was the chromaffin cell. Chromaffin cells were columnar in shape and rather basophilic. At higher magnification, they were seen to have a granular cytoplasm due to hormone-containing granules (Figure 5).

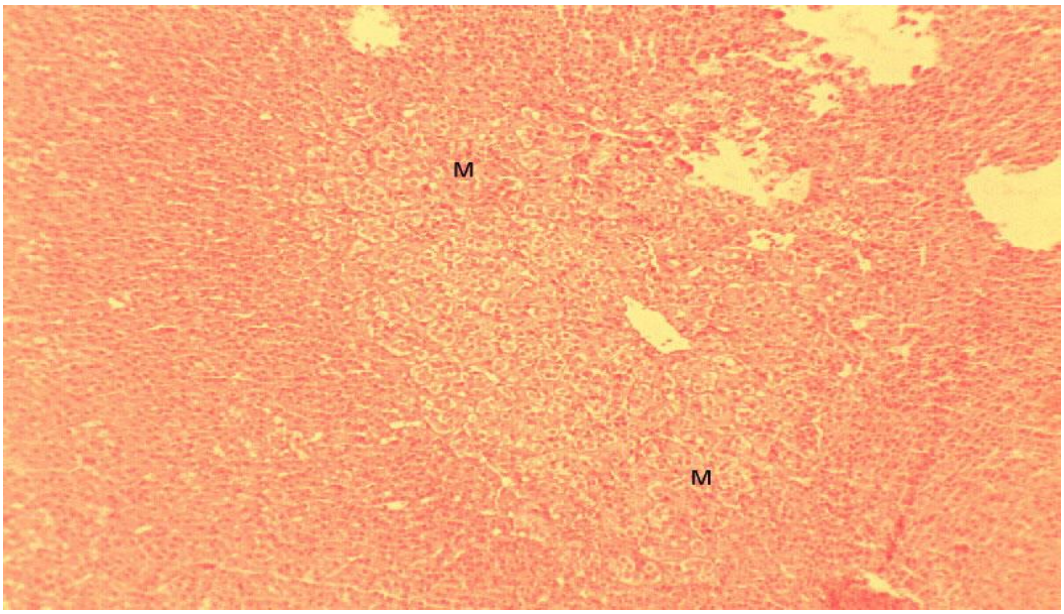

Figure 5. Light micrograph of adrenal medulla of heat stressed rats. M=medulla, H&E stain, scale bars, 100 µm.

IV. DISCUSSION

Most of the studies on the adrenal gland is associated with physiological activity (9-12). High ambient temperature induced a significant reduction of absolute and relative adrenal mass due to the reduction of cortical mass, especially that of ZF (2). Results of this study indicate that stress duration induced a significant reduction of absolute and relative adrenal mass due to the reduction of cortical mass, especially that of ZF.

There are reports suggesting that the outer part of the adrenal glands, particularly the ZG, as the source of new cortical cells, is the major site of mitosis and that most cell deaths occur within the inner part of the cortex, particularly in the ZR (13-15). It was observed that mitoses in ZG and ZF in cold stressed rats, but did not find any sign of mitoses in rat adrenal gland of heat stressed rats.

Gattermann et al. (16) stated that there were distinct sexual specific differences in both strains only for body fat and adrenal glands. In females, group housing induced an elevated level of aggression. In general, these housing conditions led to social stress symptoms, such as heavier adrenal glands. We have observed similar findings, but in males, cage density induced an elevated level of aggression.

Milosevic et al. (17) stated that in animals exposed to chronic lighting, the absolute and relative volume of zona glomerulosa was insignificantly increased by 5% compared to controls. The volume of zona glomerulosa cells and their nuclei were insignificantly changed by 1% in comparison with corresponding controls. The absolute and relative volume of zona fasciculata were significantly increased (by 14 and 9%, respectively, as compared to controls. The volume of zona fasciculata cells and their nuclei were significantly increased (by 12 and 9%, respectively. In our study, we have observed that the volume density of zona reticularis and medulla was significantly increased, and that of capsula, zona glomerulosa and zona fasciculata decreased.

The volume density of medulla was significantly increased, and that of cortex decreased. Cortical ZG was significantly decreased, and that of ZR increased, in the rats exposed to cage density. There were no differences in volume density of ZF between all groups of rats. Koko et al. (2) was also determined similar findings.

Ramachandran and Patel (18) have reported that during the breeding season adrenals showed an active condition with active adrenocortical cell columns. It has thought that increased cortical/ medullary ratio could be easily discerned in the adrenal sections. Like breeding season, Yardımoglu et al. (19) have also stated that oral contraceptive was caused a slightly active condition of adrenal glands in the treatment group. Although zona reticularis was thickened it was also degenerated.

In conclusion, stress type effects on response variables were negligible. Increasing stress duration however had profound effect on survival, growth, component of adrenal gland. Results obtained from the present study could be pertinent to further studies dealing with husbandry practices, medical applications.

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Table 1. The effects of stress type, stress duration, and sex on the adrenal glands components.

Groups				Average micrometric measurements in µm of the adrenal glands components					
ST	SD	S		C (µm)	ZG (µm)	ZF (µm)	ZR (µm)	M (µm)	CX (µm)
Group A	$GroupA_1$	F	R	18.1±2.32	35.3±3.28	327.2±11.32	112.2±12.34	251.3±34.12	474.7±11.48
			L	17.5±3.24	34.6±4.17	328.1±10.14	108.3±11.62	249.7±35.58	471±22.24
		M	R	19.7±2.54	37.6±3.43	326.2±9.21	114.1±13.37	238.6±37.15	477.9±21.09
			L	18.8±3.47	36.9±1.19	325.8±10.09	113.3±12.32	236.4±41.54	476±21.13
	$GroupA_2$	F	R	19.9±2.65	33.3±1.13	326.6±8.14	126.3±9.17	271.5±29.18	486.2±15.56
			L	19.3±3.26	33.1±2.18	324.9±11.24	125.9±10.38	269.6±28.14	483.9±17.25
		M	R	17.1±2.14	34.1±3.47	328.4±8.17	116.2±12.65	264.4±54.48	487.7±26.32
			L	16.8±3.21	33.8±2.24	327.4±9.32	115.4±11.42	264.1±43.24	476.6±23.94
Group B	$GroupB_1$	F	R	16.7±3.41	37.2±3.54	329.7±7.25	106.7±13.51	248.6±27.54	473.6±16.66
			L	16.4±2.18	35.5±2.64	328.5±8.36	104.3±14.32	242.8±34.29	468.3±8.15
		M	R	17.4±3.25	38.2±1.69	330.3±6.34	102.4±13.42	234.9±65.14	470.9±6.98
			L	15.9±1.26	37.5±2.21	329.7±7.54	100.3±14.25	231.8±62.27	467.5±7.24
	$GroupB_2$	F	R	16.8±3.40	34.4±3.17	332.1±5.19	124.8±12.24	258.6±24.34	491.3±6.92
			L	16.4±1.14	33.9±2.28	331.7±4.54	119.5±9.27	251.9±58.62	485.1±5.64
		M	R	17.1±2.16	36.2±2.35	324.4±12.27	114.8±10.29	254.3±24.36	475.4±8.54
			L	16.4±3.17	35.8±2.14	324.1±11.65	111.3±10.23	249.3±34.57	471.2±7.67
Group C		F	R	20.7±1.16	42.4±2.25	334±2.18	89.3±14.21	180.4±24.21	466.1±6.21
			L	20.4±2.32	40.9±1.47	332.4±3.14	87.4±13.54	178.6±31.18	460.7±6.32
		M	R	20.9±1.94	43.6±2.41	333.2±3.27	85.4±15.39	175.5±65.31	462.2±6.37
			L	20.8±1.17	42.7±1.96	332.7±2.28	83.3±16.32	173.5±37.41	458.7±6.43

ST = stress type, SD= stress duration, Group A= cold stressed rat, Group B= heat stressed rat, Group C= control, Group A_1-Group-B_1= acut stressed rat, Group A_2-Group B_2= chronic stressed rat, S = sex (M = male, F = Female), R=right, L=light, C =capsula, ZG=zona glomerulosa, ZF=zona fasiculata, ZR=zona reticularis, M=medulla, CX=cortex.

Table 2. The effects of stress type, stress duration, and sex on the adrenal glands components and their ratio (%).

Groups				Average micrometric measurements in µm of the adrenal glands components and their ratio					
ST	SD	S		Ratio, capsula to adrenal gland %	Ratio, ZG to cortex %	Ratio, ZF to cortex %	Ratio, ZR to cortex %	Ratio, cortex to adrenal gland %	Ratio, medulla to adrenal gland %
Group A	$GroupA_1$	F	R	2.43	7.43	68.92	23.63	63.79	33.37
			L	2.37	7.34	69.66	22.99	63.80	33.82
		M	R	2.67	7.86	68.25	23.87	64.91	32.40
			L	2.57	7.75	68.44	23.80	65.09	32.33
	$GroupA_2$	F	R	2.55	6.84	67.17	25.97	65.52	34.91
			L	2.49	6.84	67.14	26.01	62.61	34.88
		M	R	2.22	6.99	67.33	23.82	63.40	34.37
			L	2.21	7.09	68.69	24.21	62.91	34.86
Group B	$GroupB_1$	F	R	2.26	7.85	69.61	22.52	64.09	33.64
			L	2.25	7.58	70.14	22.27	64.37	33.37
		M	R	2.40	8.11	70.14	21.74	65.11	32.48
			L	2.22	8.02	70.52	21.45	65.36	32.41
	$GroupB_2$	F	R	2.19	7	67.59	25.40	64.07	33.72
			L	2.17	6.98	68.37	24.63	64.38	33.43
		M	R	2.28	7.61	68.23	24.14	63.65	34.05
			L	2.22	7.59	68.78	23.62	63.94	33.83
Group C		F	R	3.10	9.09	71.65	19.15	69.85	27.03
			L	3.09	8.87	72.15	18.97	69.83	27.07
		M	R	3.17	9.43	72.09	18.47	70.17	26.64
			L	3.18	9.3	72.53	18.16	70.24	26.56

ST = stress type, SD= stress duration, Group A= cold stressed rat, Group B= heat stressed rat, Group C= control, Group A_1-Group-B_1= acut stressed rat, Group A_2-Group B_2= chronic stressed rat, S = sex (M = male, F = female), R=right, L=light, C =capsula, ZG=zona glomerulosa, ZF=zona fasiculata, ZR=zona reticularis, M=medulla, CX=cortex.