

## Postmortem diagnosis of induced fatal hypothermia in adult albino rats

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**Abstract:** Postmortem diagnosis of fatal hypothermia is a major challenge encountered by the forensic expert because of its complex pathophysiology mechanism. This study aims to establish more specific criteria for the postmortem diagnosis of induced fatal hypothermia in rats by the assessment of serum level of cortisol, adrenocorticotrophic hormone, adrenaline, troponin1, calcium, magnesium, some free fatty acids and histopathological changes of heart, brain, anterior pituitary and adrenal gland. Sixty adult albino rats were divided into three groups; each group consists of twenty rats. The control group was kept at a normothermic condition ( $23\pm 2^{\circ}\text{C}$ ), the second group was introduced into a refrigerator at hypothermic condition ( $2-8^{\circ}\text{C}$ ) till the death while the third group was introduced into a deep freezer at hypothermic condition ( $0$  to  $-2^{\circ}\text{C}$ ) till death. The control rats were sacrificed when the rats of hypothermic groups died. Hypothermia led to significant abnormalities in the serum level of cortisol, ACTH, adrenaline, troponin 1, calcium, magnesium, palmitic and stearic acids associated with histopathological changes in brain, heart, anterior pituitary and adrenal gland according to the severity of hypothermia. Postmortem diagnosis of fatal hypothermia depends on multi-factorial criteria that include biochemical and histological abnormalities.

**Key Words:** diagnosis, hypothermia, postmortem.

Hypothermia appears when the core body temperature reaches below  $35^{\circ}\text{C}$  where the body's heat loss exceeds heat production by cold air exposure, which takes several hours to cause hypothermia or by the body immersion in water, which causes hypothermia within an hour because it draws heat away from the body faster in comparison to air [1]. Hypothermia may be intentional as a tissue preservation or therapeutic agent for neonatal hypoxic-ischemic brain injury; it slows the metabolic rate to protect the organs from the reduced oxygen supply. It may also be accidental as a result of the exposure to cold weather [2].

Hypothermia is classified into primary and secondary; the primary type occurs with an extreme cold exposure such as the cold air or water but it is associated with appropriate body heat homeostasis while the secondary

type occurs with the persons who have the impaired heat-balancing mechanism such as malnutrition and septicemia cases in old age, so they cannot respond properly to the cold exposure [3]. Diagnosis of hypothermia – induced death is still considered a major challenge for forensic experts in the temperate and cold climates because of poor understanding for the pathophysiologic mechanism of the fatal hypothermia [4].

The diagnosis of fatal hypothermia is difficult because it depends on the exclusion of other causes of death and has non-specific signs at the autopsy [5]. Thus, we need more specific and accurate criteria for diagnosis hypothermia- induced death especially in instances with accelerated cooling and circumstance, which shorten the time needed for hypothermia to occur, such as alcoholic intoxication [6]. Therefore, the current study aims to

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establish the postmortem diagnosis of induced fatal hypothermia in adult albino rats by the assessment of serum level of cortisol, adrenaline, adrenocorticotrophic hormone (ACTH), troponin I, calcium, magnesium, some free fatty acids (palmitic and stearic) and histopathological changes of heart, brain, anterior pituitary gland and adrenal gland.

## MATERIAL AND METHODS

Sixty adult albino rats, 6-8 weeks of age, weighing 200-300g were obtained from the animal house of King Abdul Aziz University- Jeddah. Rats had free access to food and water during the experimental period whether in the room, refrigerator or deep freezer. They were divided into three groups; each group comprising of twenty rats. The first group (control) was kept at a normothermic condition ( $23\pm 2^{\circ}\text{C}$ ) in the room with stabilization of the physical condition in 12 hrs day and night cycles [7]. The second group was housed in cages and introduced into a refrigerator at hypothermic condition ( $2 - 8^{\circ}\text{C}$ ) till death (the rats died in the refrigerator within 72-77 hours) [8]. The third group was housed in cages and introduced into a deep freezer at hypothermic condition ( $0$  to  $-2^{\circ}\text{C}$ ) till death (the rats died in the deep freezer within one hour). The rats from the first control group were sacrificed when the rats of hypothermic groups died. The daily measurement of the rats core body temperature was done via the rectal thermometer implantation in the rectum of rats to ensure that the first group rats were at normothermic condition ( $37^{\circ}\text{C}$ ) while the second and third group rats were at hypothermic condition (below  $35^{\circ}\text{C}$ ). The core body temperature of the second group rats was moderate hypothermic degree at  $30- 33^{\circ}\text{C}$  while the core body temperature degree of the third group rats was severe hypothermic at  $\leq 30^{\circ}\text{C}$  [9].

At the end of the experiment, after sacrifice of the rats from the first group and the death of the second and third group rats; we took tissue samples from the heart, brain, anterior pituitary gland and adrenal gland. The samples were fixed in 10% neutral buffered formalin. The fixed specimens were trimmed, washed and dehydrated in the ascending grades of alcohol, cleared in xylene, embedded in paraffin, sectioned at 4-6  $\mu\text{m}$  thickness and stained by haematoxyline and eosin according to Bancroft and Gamble [10]. An incision in the right iliac artery was carried out to get a blood sample; it was left at room temperature for 15-30 minutes to clot. The sample was centrifuged in 2000 rounds for 10 minutes at  $4^{\circ}\text{C}$  to remove the clot and separate the serum sample that was stored at  $-20^{\circ}\text{C}$  until the assay.

Measurement of Troponin I (cTnI) was done using an Elecsys analyser by the troponin I STAT third generation. This assay was based on electrochemiluminescence immunoassay technology (ECLIA) using two mouse monoclonal antibodies in a

sandwich format; it was a two step assay. The Troponin I assay was done on Elecsys 1010 and 2010 immunoassay analysers according to the manufacturer's instructions (Roche Diagnostics, Tutzing, Germany) [11].

The serum cortisol and adrenocorticotrophic hormone (ACTH) levels were isolated from the blood sample by using dichloromethane; it was determined by an ultra-high pressure Accela liquid chromatography coupled with the LCQ Advantage Max ion trap mass spectrometer (Thermo Finnigan, USA). The mass detector was operated in the atmospheric pressure positive chemical ionization mode. Chromatographic separation was performed by an adsorbosphere HS-C18 (5 mm, 250\_4.6 mm) column. The serum cortisol and adrenocorticotrophic hormone (ACTH) levels were eluted by two component mobile phase consisting of Chromasolv1 LC-MS acetonitrile (Riedel de Haen, Germany) and deionized water obtained employing the Milli-Q system (Millipore, USA) [12].

The serum adrenaline level was diluted via a saline before its measurement by using high performance liquid chromatography whereas the accuracy and reliability were checked for reproducibility following the serial 10 fold dilutions [13, 14].

The serum calcium assay was performed by calcium colorimetric assay Kit at concentration 0.4-100 mg/dL (0.1-25mM) at absorbance 575nm [15], while the serum magnesium was assayed by the calmagite dye method. Two levels of the control sera were run with every batch of the assay to ensure the accuracy and the quality assurance [16].

The free fatty acid was isolated from the blood sample by the solid phase extraction using the GX-274 ASPEC (Gilson, USA) automated system. The Merck RP- C18 (Merck, Germany) extraction columns were applied. 5 mL of deionised water was added to 0.5 mL of blood, ultrasonicated for 15 min and centrifuged at 5000 rounds per minute. The solid phase extraction columns were conditioned with 1 mL of methanol plus 2 mL of deionised water. 4 mL of blood supernatant was injected into the column. The columns were washed with 1 mL of acetic acid followed by 2 mL of deionised water and dried with nitrogen for 5 min. The free fatty acid was eluted by using 2 mL of acetone. The eluate was evaporated with the nitrogen stream at  $50^{\circ}\text{C}$  in the RapidVap concentrator (Labconco, USA). The dry residue was derivatised using 50 mL of 10% of pentafluorobenzyl bromide in acetonitrile (15 min at room temperature) with the addition of 10 mL of triethylamine as a catalyst. The solvent was evaporated; the dry residue was dissolved in 50 mL of ethyl acetate and placed in the Autosampler vials (2 mL). The use of negative chemical ionization was enabled to decrease fragmentation and form stable ions derived from the carboxylate anions. A quantifier ion and a qualifier ion were selected for every fatty acid and two internal standards [17].

### **Statistical analysis**

Statistical analysis was performed using SPSS version 17. The data was expressed as mean  $\pm$  SD and the analysis was performed by using one-way ANOVA and post-hoc multiple comparisons tests (TUKEY) to investigate the difference between the biomarker levels among the different groups where the P value of 0.05 was considered statistically significant.

### **Ethical considerations**

The most appropriate animal species were chosen for this research. Promotion of high standard care and animal well-being were exercised at all times. An appropriate sample size was calculated for using the fewest number of animals to obtain the valid results statistically. Painful procedures were performed under anesthesia to avoid any distress and pain that could be inflicted on the animals. Our standards of the animal care are consistent with the requirements and standards of international laws and regulations.

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## **RESULTS**

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### **Biochemical findings**

Table (1) shows the effects of different degrees of induced hypothermia on Mean  $\pm$  SD of different biomarkers in the rats. Mean  $\pm$  SD values of different hormones (Cortisol, ACTH and Adrenaline) are increased significantly in the third severe hypothermic group (0 to  $-2^{\circ}\text{C}$ ) in comparison to the second moderate hypothermic group (2 -  $8^{\circ}\text{C}$ ) that is increased significantly in comparison to the first normothermic group at  $P < 0.001$ . Mean  $\pm$  SD values of cardiac enzyme and electrolytes (Troponin 1, Calcium, and Magnesium) are increased significantly in the third severe hypothermic group (0 to  $-2^{\circ}\text{C}$ ) in comparison to the second moderate hypothermic group (2 -  $8^{\circ}\text{C}$ ) that is increased significantly in comparison to the first normothermic group at  $P < 0.001$ . Mean  $\pm$  SD values of some free fatty acids (Palmitic and Stearic) are decreased significantly in the third severe hypothermic group (0 to  $-2^{\circ}\text{C}$ ) in comparison to the second moderate hypothermic group (2 -  $8^{\circ}\text{C}$ ) that is increased significantly in comparison to the first normothermic group at  $P < 0.001$ .

### **Histopathological findings**

#### *A-Cardiac histopathological findings*

The examination of cardiac sections in the rats of the first normothermic group revealed a normal structure of myocardium (Fig. 1). The cardiac tissues in the rats of the second moderate hypothermic group (2 -  $8^{\circ}\text{C}$ ), showed a moderate disturbance in the cardiac muscle architecture, fragmentation and degeneration of the myocardial fibers, areas of necrosis with pyknotic nuclei and hemorrhage, sporadic vacuoles in the different areas of myocardium, cardiac valves and coronary vessels (Fig. 2). The cardiac

sections in the rats of the third severe hypothermic group (0 to  $-2^{\circ}\text{C}$ ) showed a severe disturbance in the cardiac muscle architecture with massive areas of necrosis and a severe vacuolation in myocardium beside pyknotic nuclei and hemorrhage (Fig.3)

#### *B- Brain histopathological findings*

The examination of brain tissues in the rats of the first control normothermic group showed a normal brain structure (Fig. 4), but the brain tissues in the rats of the second moderate hypothermic group (2 -  $8^{\circ}\text{C}$ ) showed a loss in the normal architecture, pyramidal and glial cells degeneration with hemorrhagic areas and vacuoles (Fig. 5). Moreover, the brain tissues in the rats of the third severe hypothermic group (0 to  $-2^{\circ}\text{C}$ ) showed a marked disturbance in the brain architecture, widespread of pyramidal and glial cells degeneration, pyknotic nuclei with massive areas of hemorrhage and vacuoles (Fig. 6).

#### *C-Adrenal gland histopathological findings*

The examination of adrenal glands in the rats of the first control normothermic group showed the normal structure of adrenal gland (Fig. 7). Whereas, the adrenal glands in the rats of the second moderate hypothermic group (2 -  $8^{\circ}\text{C}$ ) showed chromaffin cells congestion, some vacuoles and congested capillaries (Fig. 8). In addition to this, the adrenal gland in the rats of the third severe hypothermic group (0 to  $-2^{\circ}\text{C}$ ) showed a marked loss in the normal architecture, widespread of degenerated chromaffin cells with huge areas of vacuoles and hemorrhage (Fig. 9).

#### *D-Anterior pituitary gland histopathological findings*

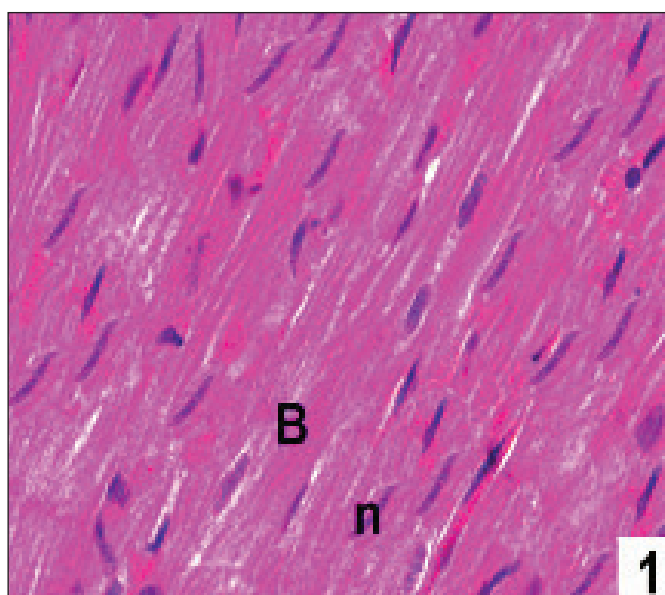
The examination of anterior pituitary gland in the rats of the first control normothermic group showed normal structure of anterior pituitary gland (Fig.10). While the anterior pituitary gland in the rats of the second moderate hypothermic group (2 -  $8^{\circ}\text{C}$ ) showed a vacuolated cytoplasm in some cells of Pars distalis, perinuclear halo with congested and dilated capillaries in other cells of Pars distalis (Fig. 11). The anterior pituitary gland in the rats of the third severe hypothermic group (0 to  $-2^{\circ}\text{C}$ ) showed a marked loss in the normal architecture of Pars distalis, widespread of degenerated chromophilic cells with pyknotic nuclei and extensive areas of hemorrhage (Fig. 12).

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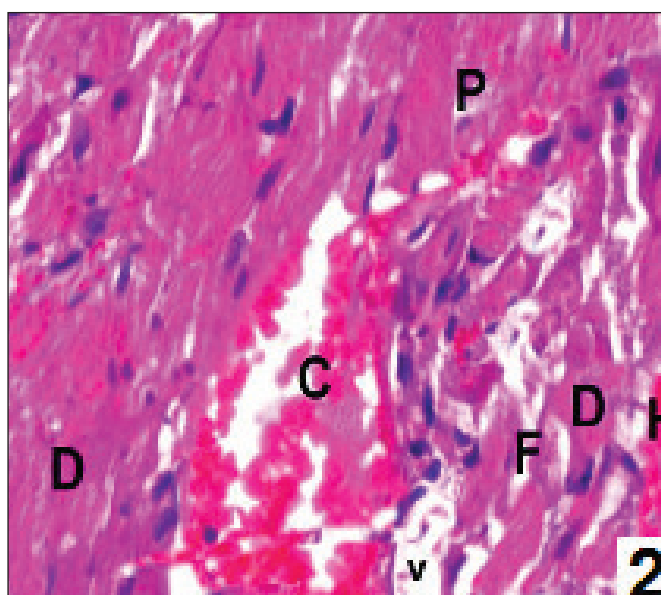
## **DISCUSSION**

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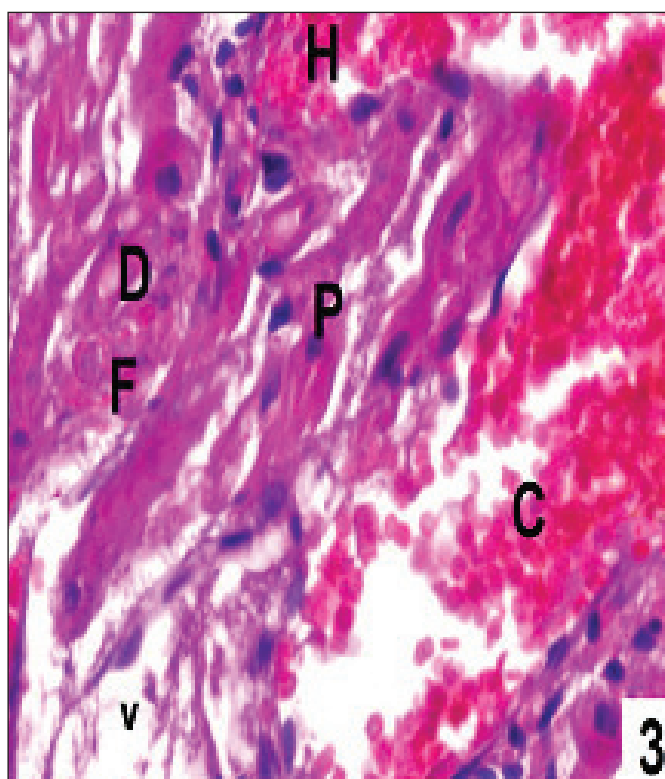
Investigation of the fatal hypothermia cases is very difficult issue in the forensic medicine because the only histopathological changes are not usually enough for postmortem diagnosis of hypothermia despite the fact that forensic pathology advanced significantly in the last years. So, the diagnosis of hypothermia fatalities becomes limited and mainly depends on the exclusion of other causes of the death. Our study aims to set the diagnostic criteria for fatal hypothermia based



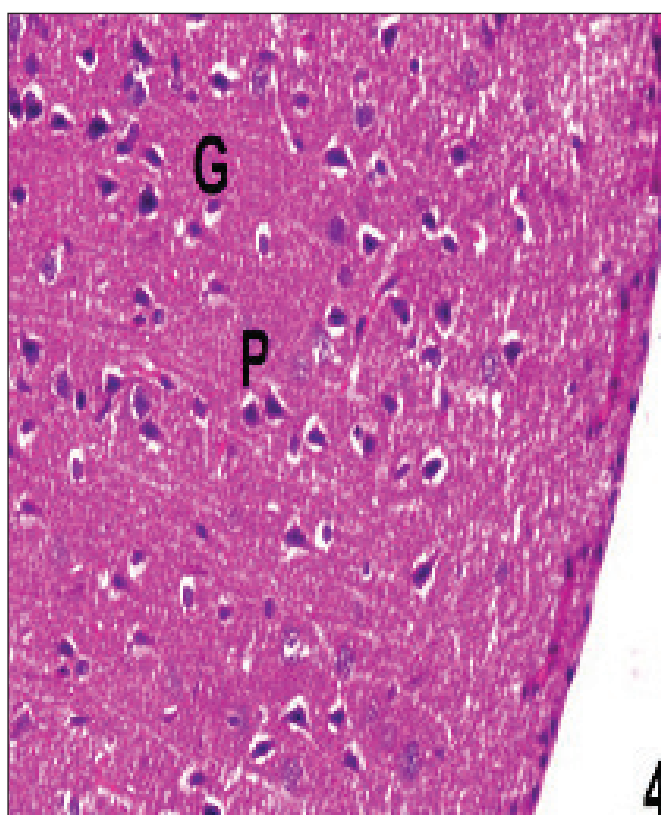
**Figure 1.** A photomicrograph of transverse section in the control normothermic rat heart shows normal branching and anastomosis cardiac muscle fibers (B), central elongated nuclei (n) and acidophilic cytoplasm (H&E X400).



**Figure 2.** A photomicrograph of transverse section in the second moderate hypothermic group rat heart shows a loss in the cardiac muscle fibers (F), areas of necrosis with pyknotic nuclei (P), necrotic areas without nuclei (D), vacuoles (v), hemorrhage (H) and congested coronary blood vessels (C)(H&E X400).



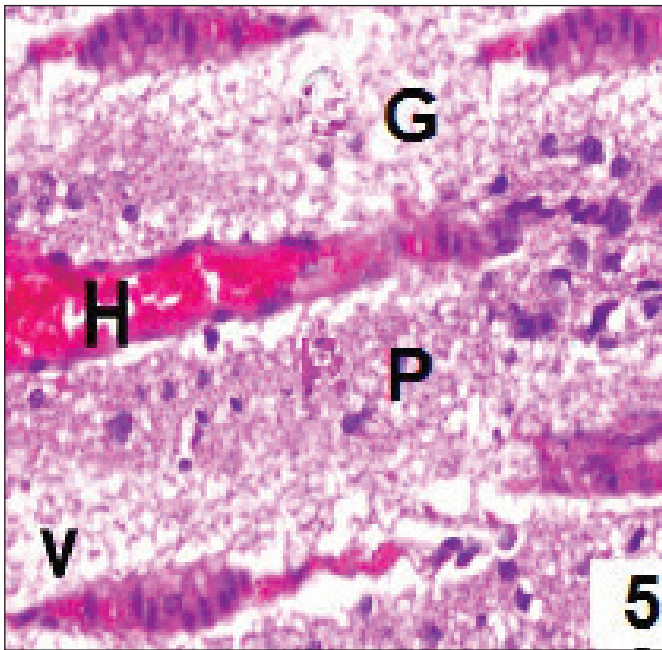
**Figure 3.** A photomicrograph of transverse section in the third severe hypothermic group rat heart shows marked loss of cardiac muscle fibers (F), pyknotic nuclei (P) with wide areas of vacuoles (v), extensive necrotic areas without nuclei (D), hemorrhagic areas (H) and congested coronary blood vessels (C) (H&E X400).



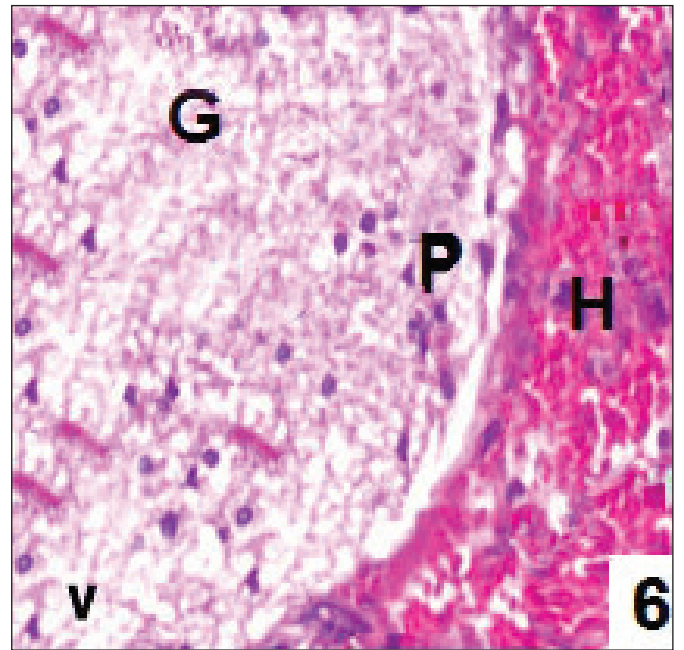
**Figure 4.** A photomicrograph of transverse section in the control normothermic rat brain shows normal pyramidal cells (P) and glial cells (G) (H&E X400).

on biochemical and morphological parameters. The current study demonstrated that there is a statistical significant increase in the hormones levels (Cortisol, ACTH and Adrenaline) in the second moderate hypothermic group that are more increased as a sequel of excessive lowering of body temperature in the third

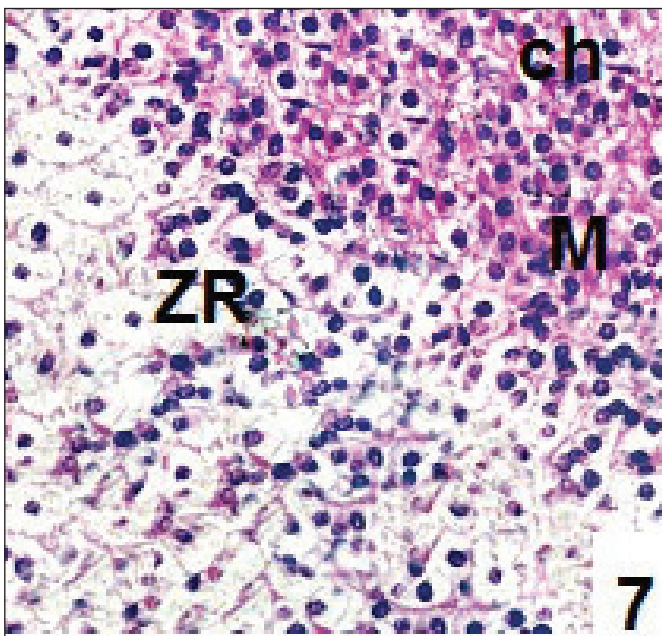
severe hypothermic group. This is in consistent with [18, 19] who showed that hypothermia stimulates the hypothalamic–pituitary–adrenal axis leading to an increase in the levels of adrenaline, cortisol and ACTH as a compensatory mechanism although the elevated levels of these hormones are not considered reliable



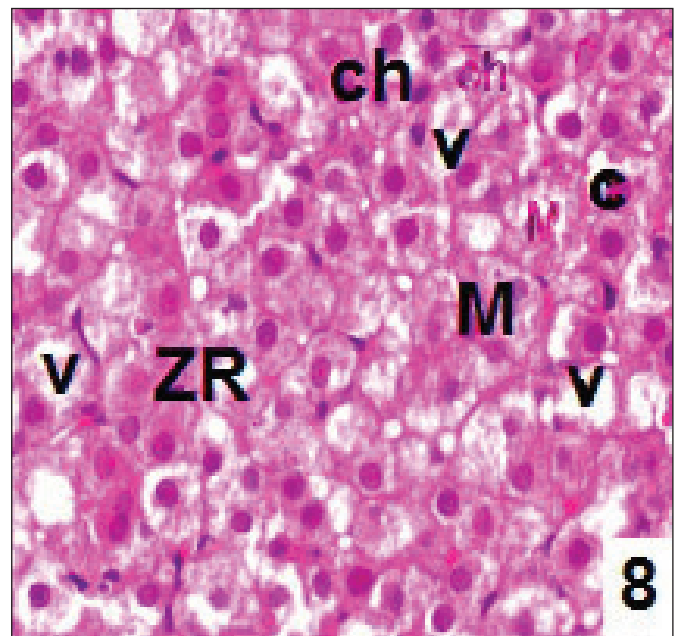
**Figure 5.** A photomicrograph of transverse section in the second moderate hypothermic group rat brain shows a loss in the normal brain architecture, degenerated pyramidal cells (P) and glial cells (G), vacuoles (v) and hemorrhage (H) (H&E X400).



**Figure 6.** A photomicrograph of transverse section in the third severe hypothermic group rat brain shows a marked loss in the brain architecture, marked degeneration of pyramidal cells (P) and glial cells (G), huge vacuoles (v) and hemorrhage (H) (H&E X400).



**Figure 7.** A photomicrograph of transverse section in the control normothermic rat adrenal gland shows normal zona reticularis (ZR) and chromaffin cells (ch) that are arranged in anastomosing cords adjacent to the capillaries and adrenal medulla (M) (H&E X400).

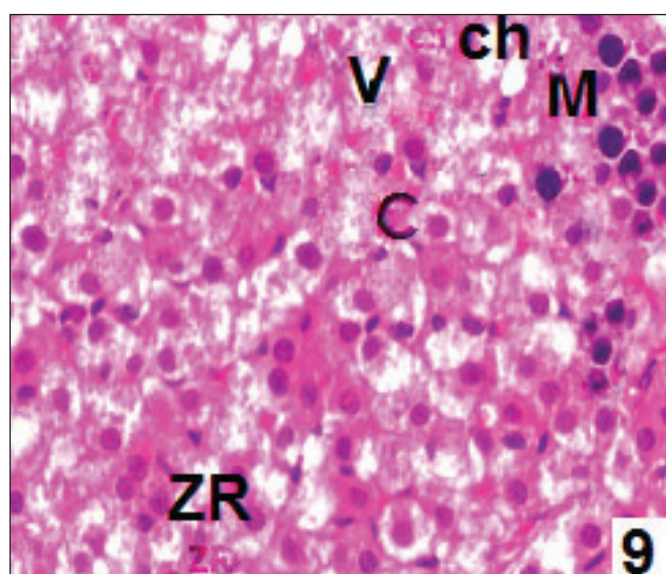


**Figure 8.** A photomicrograph of transverse section in the second moderate hypothermic group rat adrenal gland shows congested chromaffin cells (ch) that are surrounded by congested capillaries (c), some vacuoles (v), adrenal medulla (M) and zona reticularis (ZR) of adrenal cortex (H&E X400).

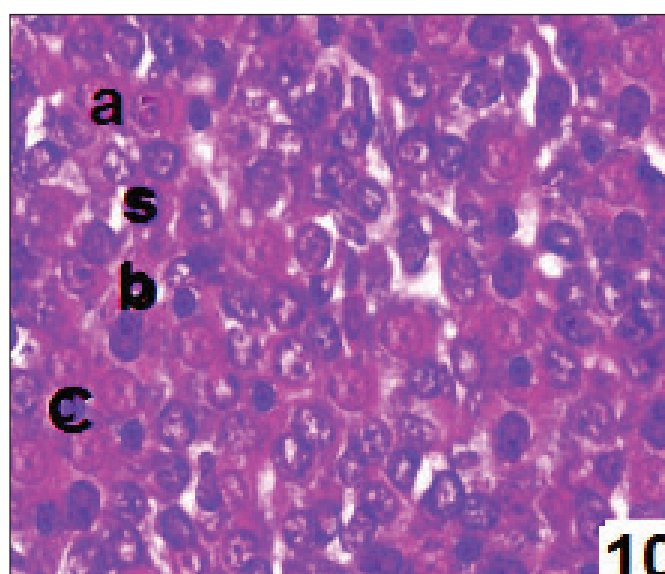
markers of hypothermia - induced death according to Palmiere and his colleague [20] who referred that it may be discovered also in the death that may be attributed to other causes than hypothermia. Conversely, Castellani *et al.*, [21] and Ma and Morilak [22], confirmed that the cortisol, ACTH and adrenaline can be used as fatal hypothermia markers.

Our results showed a statistical significant

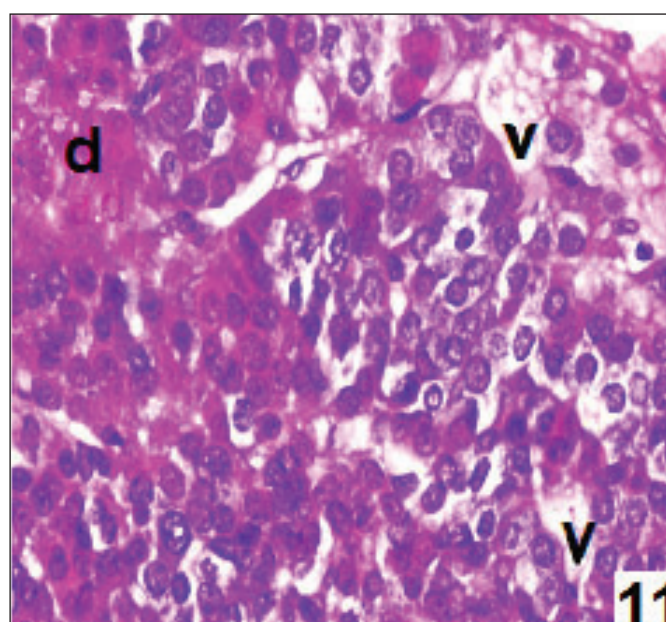
increase of troponin1, calcium, and magnesium levels in the second moderate hypothermic group that are more increased in severe hypothermic group in comparison to the second group. This is in consistent with Turk [5], who showed that the cardiac failure and electrolytes disturbance are the most causes of death in hypothermia due to the elevated myocardial oxygen consumption because of the sympathetic over-activity leading to



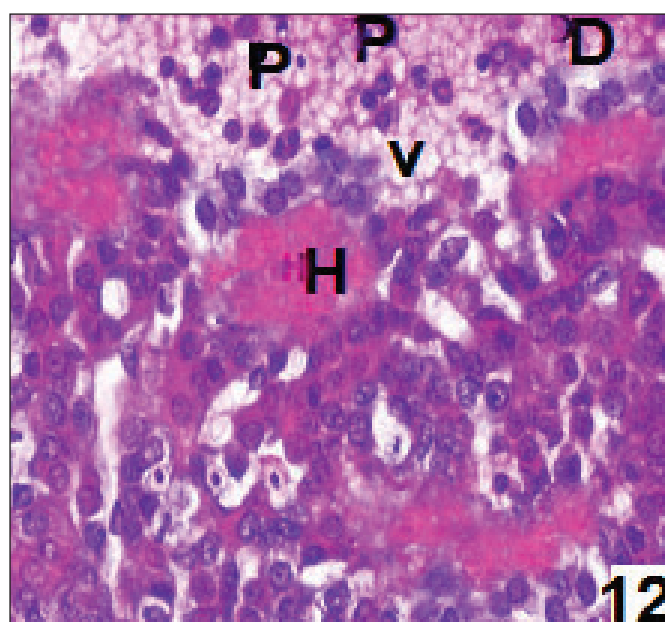
**Figure 9.** A photomicrograph of transverse section in the third severe hypothermic group rat adrenal gland shows a marked loss in the adrenal gland architecture, degenerated chromaffin cells (ch), congested capillaries (c) and hemorrhage, more vacuoles (v), adrenal medulla (M) and zona reticularis (ZR) of adrenal cortex (H&E X400).



**Figure 10.** A photomicrograph of transverse section in the control normothermic rat anterior pituitary gland shows normal pars distalis and chromophobe cells (c), normal acidophilic chromophilic (a) and basophilic cells (s) that are separated by thin walled sinusoids (b) (H&E X400).



**Figure 11.** A photomicrograph of transverse section in the second moderate hypothermic group rat anterior pituitary gland shows vacuoles (v) in the cytoplasm of some Pars distalis cells, perinuclear halo with congested and dilated capillaries (d) in other cells of Pars distalis (H&E X400).



**Figure 12.** A photomicrograph of transverse section in the third severe hypothermic group rat anterior pituitary gland shows a marked loss in the normal architecture, widespread of degenerated areas (D), degenerated of chromophilic cells with pyknotic nuclei (p), huge areas of vacuoles (v) and hemorrhage (H) (H&E X400).

the high level of troponin1 that is highly specific for acute myocardial infarction according to Jeremias and Gibson [23]. Hypothermia causes a loss in the ion regulation leading to electrolytes disturbance that may affect the cardio-respiratory function as a result of the inotropic response to cooling. This in turn causes an increase in the calcium release via action potential with reducing the calcium influx into the left atria of heart.

According to Li *et al.* [24], there is a disturbance in the levels of calcium and magnesium in the cases of fatal hypothermia. Conversely, these findings are in contrast with Jakubeniene *et al.* [25], who confirmed that there are not any significant differences in the calcium and magnesium levels in cases of fatal hypothermia in comparison to the cases of death in the normothermic condition in agreement with Palmiere *et al.* [26].

**Table 1.** Comparison between the effect of different degrees of induced hypothermia on Mean  $\pm$  SD of different biomarkers in the rats

Group Parameter	First M $\pm$ S.D	Second M $\pm$ S.D	Third M $\pm$ S.D	F
Cortisol (ug/dL)	15.692 $\pm$ 1.62	27.81 $\pm$ 0.90*	32.385 $\pm$ 1.75**	680.469
ACTH (pg/mL)	10.745 $\pm$ 0.46	14.345 $\pm$ 1.29*	17.38 $\pm$ 0.64**	287.466
Adrenaline (ng/L)	34.415 $\pm$ 10.50	67.14 $\pm$ 2.48*	75.465 $\pm$ 1.84**	235.471
Troponin 1 (ng/mL)	0.0028 $\pm$ 0.001	0.762 $\pm$ 0.0143*	0.872 $\pm$ 0.07**	2577.475
Calcium (mg)	11.78 $\pm$ 1.04	15.435 $\pm$ 0.88*	18.57 $\pm$ 0.75**	282.764
Magnesium (mg)	4.095 $\pm$ 0.81	7.03 $\pm$ 0.58*	10.62 $\pm$ 0.95**	335.177
Palmitic acid ( $\mu$ Eq/L)	565.4 $\pm$ 38.55	691.15 $\pm$ 22.70*	424.3 $\pm$ 14.69**	482.134
Stearic acid ( $\mu$ Eq/L)	51.755 $\pm$ 0.57	64.03 $\pm$ 2.21*	41.86 $\pm$ 0.718**	1287.246

Number per group: 20; ACTH: Adrenocorticotrophic hormone

M $\pm$ S.D: Mean  $\pm$  Standard Deviation. F: The value of the difference between the groups.

First group (control) is kept at a normothermic condition (23 $\pm$ 2°C).

Second group is introduced into a refrigerator at hypothermic condition (2 - 8°C).

Third group is introduced into a deep freezer at hypothermic condition (0 to -2°C).

\* P < 0.001 (significant difference in comparison to the first group)

\*\* P < 0.001 (significant difference in comparison to the second group).

The present study referred that palmitic and stearic free fatty acids are increased significantly in the second moderate hypothermic group in comparison to the first normothermic group in consistent with [18, 20], who showed that hypothermia induces an increase in the adrenal hormones due to the sympathetic-adrenal system activation and post-mortem autolysis to release the fatty acid molecules to the blood leading to the rising of palmitic and stearic acids levels as one of the mechanisms of maintaining heat homeostasis according to Leppaluoto *et al.* and Haman *et al.* [27, 28]. But, our results reported that palmitic and stearic acids levels are decreased significantly in the third severe hypothermic group in comparison to the second moderate hypothermic group because of the reduction of lipolysis in severe hypothermia.

This study demonstrated that there is a statistical significant increase of troponin1 level and cardiac histopathological changes such as fragmentation and degeneration of the myocardial fibers, areas of necrosis with pyknotic nuclei and hemorrhage, sporadic vacuoles in the different areas of myocardium and coronary vessels in the second moderate hypothermic group. These remarkable cardiac histopathological findings become more severe in the third severe hypothermic group with more statistical significant increase of troponin1 level in comparison to the second moderate hypothermic group. These results confirm the findings of Preuss *et al.* and Furukawa *et al.* [29, 30], who showed the presence of myocardial necrosis and fatty vacuoles in cardiomyocytes in cases of fatal hypothermia leading to myocardial infarction due to hypoxic cardiac cells that result from the cooling of heart where the high increase of troponin1 level is considered a biomarker for the occurrence of myocardial infarction. Hypothermia usually decreases the oxygen release from hemoglobin into the tissue because of shifting the oxy-hemoglobin-dissociation

curve to the left causing the cell death in accordance with Shutt and his colleague [31].

Our results showed anterior pituitary gland histopathological changes in the rats of the second moderate hypothermic group such as the vacuolated cytoplasm with congestion and dilation of capillaries in some cells of Pars distalis, but these changes become more severe in the rats of the third severe hypothermic group such as the loss of normal architecture of Pars distalis beside the widespread of degenerated chromophilic cells with pyknotic nuclei and extensive areas of hemorrhage. These results are similar to the ones reported by Ishikawa *et al.* and Doberentz *et al.* [32, 33], who showed that tissue hypoxia as a result of hypothermia leads to histopathological changes in the anterior pituitary gland based on the severity and duration of hypothermia in contrast with Klenerova *et al.* [34], who suggested that the metabolic dysfunction due to hypothermia may be the cause of histological alterations in the anterior pituitary gland especially vacuoles.

The current study showed brain histopathological changes in the rats of the second moderate hypothermic group such as the disturbance of normal architecture, pyramidal and glial cells degeneration with hemorrhagic areas and vacuoles that are more severe in the rats of the third severe hypothermic group. These findings result from a direct cell damage due to the changes in the microcirculation such as vasoconstriction and the formation of intravascular sludge that are aggravated in severe hypothermic condition where the freezing of extracellular fluid and the cell damage determine the extent of injury according to Koljonen *et al.* [35] and Muldrew and McGann [36]. In the same context, Park *et al.* [37], reported that the glia cells are the most plentiful cell population in the brain; it surrounds and protects the neurons because it has a rapid activation in response to a variety of stimuli but its number is decreased

during the cold exposure depending on the severity of hypothermia beside the decrease in neuropeptide Y that is localized in the brain and plays a role in thermoregulation. On contrary, Polderman [38] referred that hypothermia protects the brain against the injury by the number of cellular mechanisms such as the reduction in mitochondria dysfunction and cell metabolism, prevention of apoptosis and the suppression in radical oxygen species production.

Regarding the adrenal glands, there are many histopathological changes in the rats of the second moderate hypothermic group such as chromaffin cells and capillaries congestion that converted into the degenerated cells with areas of vacuoles and hemorrhage in the rats of the third severe hypothermic group. These histopathological findings result from the hypothalamic-pituitary-adrenal axis activation and ACTH production that is increased to generate the heat, then it is suppressed because of the cooling progress which leads to significant metabolic disorders resulting in the lipid mobilization

from the tissue stores and then fatty vacuoles formation in accordance with McRae *et al.* [4]. In other context, Madea *et al.* [39] showed that morphological findings and their pathogenesis in the different organs in the cases of fatal hypothermia occur only with the temperature below zero as a sequel of the cell injury due to the changes in the circulation during the cold exposure that are aggravated by the cold agglutinins.

In conclusion, postmortem diagnosis of fatal hypothermia depends on multi-factorial criteria that include biochemical and histological abnormalities such as the disturbance in the levels of hormones, free fatty acids, electrolytes and cardiac enzyme associated with morphological changes in heart, brain, anterior pituitary and adrenal gland that vary its severity according to the degree of cooling. Further research in human is recommended to verify our results.

**Conflict of interest.** Authors declare that there is no conflict of interest.

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