Histological, Histochemical and Biochemical Changes in the Liver, Kidney, Lung and Spleen under the Effect of Repetitive Hyperthermia in Rat Neonates

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Abstract

Background: Artificial hyperthermia in association with radiotherapy or chemotherapy has become a promising method for cancer treatment. Thus, this study was designed to assess the impact of repetitive hyperthermia (41° ± 0.5°C) on some normal histological, histochemical, and biochemical variables in newborn rats.

Materials and methods: After parturition, the neonates were randomly chosen and assigned to two groups (each being comprised of ten animals): one group was exposed to hyperthermia (41° ± 0.5°C) and the other one was exposed to normothermia (25° ± 0.5°C). Both groups were treated daily for 2 hours from the day of labor until the age of 21 days.

Results: Histological and histochemical examination of the tissue sections of hypothermic rat neonates exhibited numerous cirrhotic changes in liver with deposition of collagen fibers extending from the central veins or portal tracts forming thick or thin fibrotic septa and even pseudolobule formations. The kidneys showed areas of necrosis, edema, glomerular hyperplasia or infiltration of inflammatory cells, marked amounts of collagen surrounding some Malpigian corpuscles and in between the renal tubules in a focal or diffuse fashion. The lungs revealed alveolar haemorrhage, focal fibrosis, emphysema of the alveoli with rupture of some alveolar walls and hyperplasia of the cells lining alveoli with collagen distribution in the peribronchiolar area as well as in the tunica adventitia of the peribronchiolar dilated blood vessels, and in the interalveolar and perialveolar areas. The spleen suffered from severe hyperemia in the red pulps and sinusoids with distorted lymphoid nodules and atrophy of others. The quantitative measurement of the degree of fibrosis proved a significant ($p < 0.01$) accumulation of collagen fibers in all the examined tissues except for spleen.

Additionally, hyperthermia caused a significant increase in most measured serum biochemical variables (ALT, AST, GGT, LDH, total bilirubin, total protein, albumin, creatinine & urea) except for the globulin content whose concentration showed a significant decrease.

Conclusion: As high preferential absorption ability of tumour tissue components to heat over normal tissues rely, in part, upon their higher collagen and protein content, attention should be paid to the repetitive effect of high temperature on the architecture of normal tissues, total collagen and protein contents in addition to its upshots on certain biochemical indices to appraise the impact of the method on patients particularly newborns and those with multi-recurrences carcinoma.

Keywords: hyperthermia, histopathology, histochemistry, biochemistry, rat newborns

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Introduction

Hyperthermia (HI) refers to abnormal high body temperature. It most often occurs spontaneously from fever due to illness, extremely heavy exercise or prolonged exposure (longer than 10 min) to heat sources as hot tubs, very hot baths, or saunas. Also, hyperthermia has been used in clinical settings either alone or as an adjunct to radio- and/ or chemotherapy for the treatment of various malignant diseases where it can be produced either locally or over the whole body [1-3].

Whole body (systemic) hyperthermia is specially used to treat metastatic cancers that have spread throughout the body. This can be accomplished by several techniques that raise the body temperature to 107-108 °F including the use of thermal chambers similar to large incubators or hot water blankets [4].

The biological effects of hyperthermia have been studied in numerous animal models including mice, rats, dogs, ewes, swines, rabbits and human beings [5-15]. In in vitro and in vivo experiments, HI induces a variety of histopathological changes in central nervous system (CNS), lungs, retina, placenta, kidneys, subcutaneous adipose tissue and skeletal muscles [6-7, 14-17]. Also, the increase in body temperature causes many physiological and metabolic responses in the body [18] where respiratory alkalosis, decreased total blood flow to uterus and tumours, increased GOT & GPT release, increased glycogen breakdown and glucose turnover and enhanced lipid peroxidation have all been reported [9, 19-21].

Furthermore, acute and chronic heating of cells and tissues induces alterations of nuclear and cytoskeletal structures, decrease of mitotic figures in the epithelium and somites, disruption of neural and vascular basement membranes, increase of programmed cell death, i.e., apoptosis and inhibition of natural cell-mediated immunity [22-25].

Both clinical and experimental investigations have shown that maternal hyperthermia during clinical stages of embryo development results in developmental malformations in the offspring of different animal species [13, 15, 26]. In all species studied, the CNS appeared to be at a great risk of damage [27-28]. However, It is thought that hyperthermia during pregnancy can also cause embryonic death, abortion, growth retardation and a wide range of teratogenic abnormalities including anencephaly/exencephaly, talipies, spina bifida, anophthalmia, arthrogryposis and cardiovascular malformations in conjunction with other skeletal abnormalities [28-29].

The temperature regulation in newborn mammals has been the subject of experimental research for over 50 years. Yet, it is still poorly understood. Additionally, it is thought that the proven efficacy of HI in tumour cell killing may be able to affect the normal tissues adjacent to the treated tumour or many normal tissues in the case of whole body hyperthermia, especially when the treatment is repeated. Therefore, this study was designed to assess the impact of the repetitive hyperthermia (41° ± 0.5°C) on some histological, histochemical and biochemical variables in 21-day-old newborn rats.

Materials and Methods

Animals

Adult male and female albino rats, Rattus rattus, supplied by the National Research Institute of Ophthalmology, Giza, Egypt were placed in polypropylene cages with stainless steel lids and maintained in controlled conditions of light (12hr light, 12 hr dark) and temperature (25° ± 0.5°C) in an air-conditioned room. Food and water were provided ad libitum throughout the study.

Nulliparous females (1-2) were allowed to mate with a proven male for two consecutive days. Copulation was confirmed by the presence of sperms in vaginal smears. The pregnant females were then transferred into separate cages till delivery.

After parturition, the neonates were chosen randomly and assigned to two groups (each being comprised of ten animals): one group was exposed to hyperthermia (41° ± 0.5°C) and the other one was exposed to normothermia (25° ± 0.5°C).

Experimental protocol

Induction of hyperthermia was achieved by placing the animals of the treatment group in a good aerated incubator at (41° ± 0.5°C) for 2 hours daily until the day 21 postpartum. Similarly, a group of normal neonates was exposed to normothermia (25° ± 0.5°C) for the same period of exposure until the same age. To minimize the circadian variations, animals were exposed to heat between 8.00 and 10.00 am and temperature was monitored using a body thermistor probe. Also, to avoid the stress of bereavement, the newborns were returned to their nursing mothers after each exposure.

On the day 22 of parturition, all rats were sacrificed under mild diethyl ether anaesesthia and small pieces (1mm3) of liver, kidneys, lungs and spleen were placed in neutral buffered formalin solution for 24 hours. The specimens were then processed up to paraffin blocks and sectioned at a thickness of 5 µm. Paraffin sections were stained with either haematoxylin and eosin (H & E) for a routine histological examination or Masson's trichrome for histochemical quantitative measurement of collagen content and distribution and for the estimation of the degree of fibrosis.

The degree of fibrosis was measured quantitatively by LEICA QWIN software for image analysis (Leica Imaging Ltd., Cambridge, England). Using a 40X objective lens, 20 randomly selected fields per each section were examined. Three sections for each animal were tested and the fraction of blue to green stained collagen areas was averaged for each animal. Six animals for each

group were exposed to the examination. Data was presented as percentage.

Blood samples were obtained from the cervical vein into clean, dry and labelled tubes. Blood samples were left to coagulate and then centrifuged at 3000 rpm for 15 minutes. The clear non-haemolysed sera were stored in a deep freezer at -40˚C until being used for biochemical evaluation. Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured according to the method of Bergmeyer et al. [30] using reagent kits purchased from Spin react company, while gamma glutamyl transferase (γGT) activity was evaluated by the kits obtained from Biosystems company (Spain) using the methods of Tietz [31] and Young [32]. Also, lactate dehydrogenase activity (LDH) was measured according to the method of Bűhl and Jackson [33] using the reagent kits obtained from Stanbio Laboratories (Texas, USA) and total bilirubin concentration was measured according to the procedure of Jendrassik and Grap [34] using the reagent kits obtained from Diamond Diagnostics, Egypt. Total proteins and albumin contents were assessed according to Rojkin et al. [35-36] using Weiner Laboratory reagent kits (2000-Rosario, Argentina). Moreover, creatinine, urea and uric acid concentrations were estimated by the methods of Houot [37-39], respectively.

All data was statistically analyzed applying the students't' test [40].

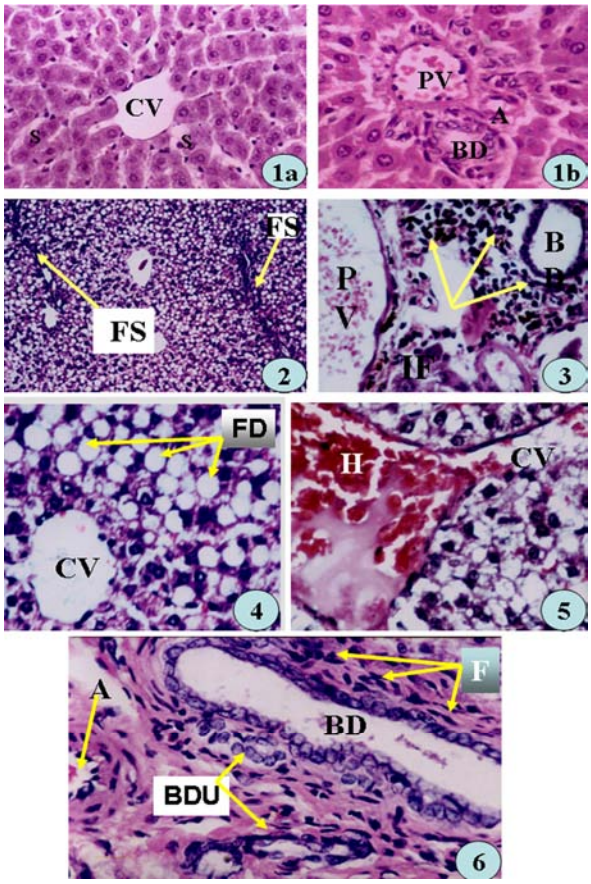
Results

1- Histopathological and histochemical findings:

The liver

The liver of the control group was composed of a number of lobules. Each lobule had a thin walled central vein from which cords of hepatic cells radiated towards the lobule periphery. The hepatic cords alternated with narrow irregular blood spaces i.e., the hepatic sinusoids (Fig.1a). Around the periphery of each lobule, branches of hepatic artery, hepatic portal vein and bile duct were present (Fig.1b).

In the heat-treated group, the hepatic cords became distorted and the hepatocytes were bloated with large, spherical droplets of fat in a focal and/ or diffuse manner (Fig. 2) in association with the dilatation and congestion of the central and portal veins and infiltration of the parenchyma with inflammatory cells (Fig. 3). These droplets pressed the nucleus toward the periphery giving a signet-ring appearance to the cell (Fig. 4). Rarely, homogeneous eosinophilic materials were also seen in the lumina of these veins (Fig. 5) while the bile ductules showed



Figs. 1, 2, 3, 4, 5 & 6 are representative photomicrograghs of liver sections stained with haematoxylin and eosin.

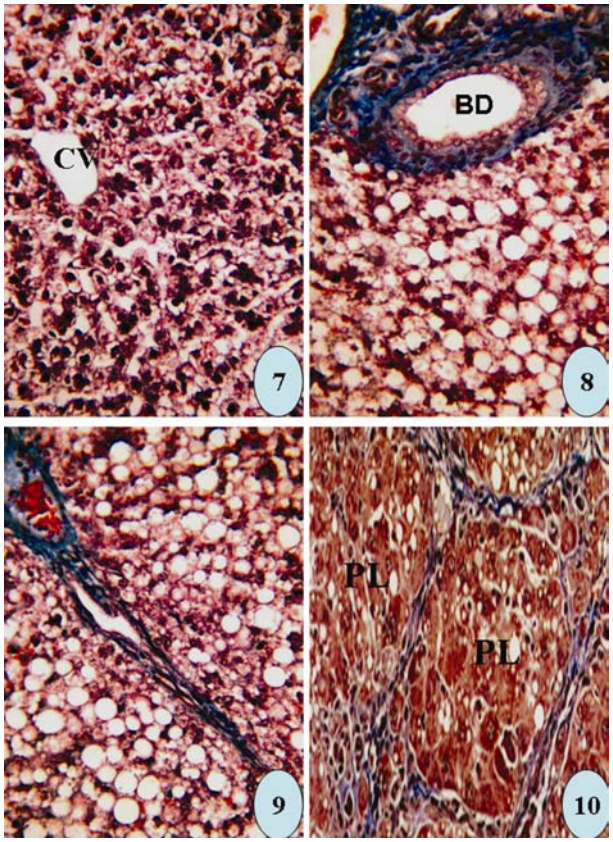
***Fig. (1a & b)**: The normal histological structure of hepatic tissue showing a thin walled central vein (CV) from which cords of hepatic cells radiate. These cords alternate with the hepatic sinusoids (S). Branches of the hepatic portal vein (PV), hepatic artery (A) and bile ductules (BD) forming the portal triads are also seen. (X512) **Fig. (2)**: The distorted liver architecture and thin fibrotic septa (FS) seen in the liver of hyperthermic rat newborns. (X128) **Fig. (3)**: Inflammatory cells (IF) infiltrating the portal area of the liver of neonates after two hours of hyperthermia exposure. (X512) Fig. (4): Fatty degenerative changes (FD) of the hepatocytes seen around the central vein (CV) with their characteristic signet-like appearance. (X512)*

***Fig. (5)**: Dilated central vein (CV) engorged with blood cells (H) and some albuminous material. (X512)*

***Fig. (6)**: Distended bile ductule (BD) and newly formed ones (NBD) surrounded by a large number of fibroblasts (F) and many necrotic areas (N). (X512)*

distention and proliferation. Also, necrosis of the hepatocytes was mainly noticed around the portal area (Figs. 2, 6). The use of Masson's trichrome stain

showed sparse collagenous supporting tissues (Fig. 7) in the parenchyma and the portal area of the normothermic rat neonates while massive deposition of collagen fibres as thick or thin fibrotic septa were seen extending between the hepatocytes from the central veins or the portal tracts, forming pseudolobules (Figs. 8, 9 & 10).

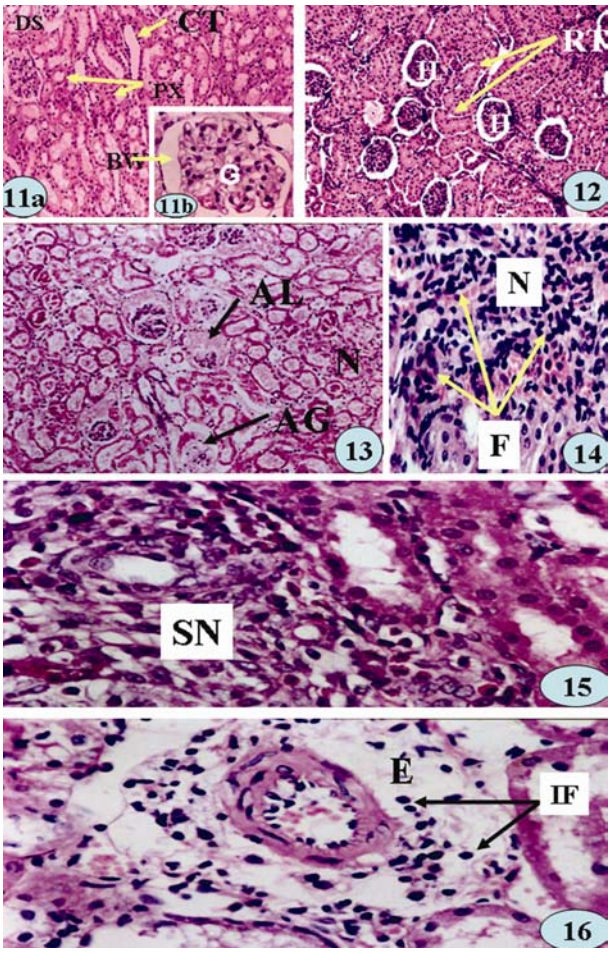


Figs. 7, 8, 9 & 10 represent collagen distribution (Blue green color) in Masson's trichrome stained liver-sections of normothermic rat newborns (Fig. 7) and hyperthermic ones (Figs. 8-10) forming pseudolobules (PL). (X512)

The kidney

The kidney cortex of the control group exhibited normal structure of renal corpuscles, renal tubules (proximal and distal) and collecting ducts (Fig. 11a). The renal corpuscle is typically formed by a tuft of blood capillaries (the glomerulus), surrounded by a Bowman's capsule (Fig. 11b).

On the other side, the kidney sections of the heat-exposed neonates exhibited some hyperplastic glomeruli and swollen lining epithelium of the renal tubules with obliterated lumina (Fig. 12). Also, periglomerular albuminous material deposition with edema and infiltration of inflammatory cells (Figs. 13, 14 & 16), were observed in association with



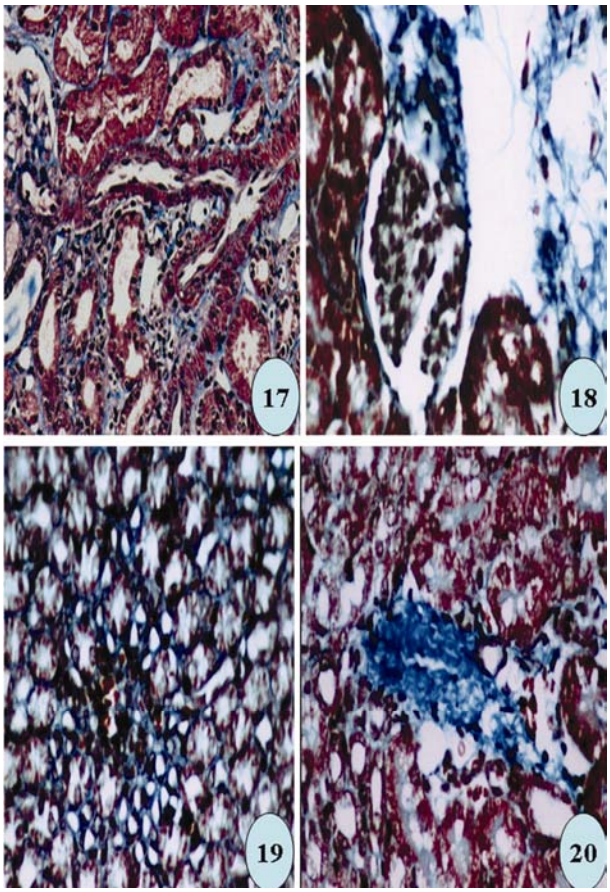
Figs. 11, 12, 13, 14, 15 & 16 are representative photomicrograghs of kidney sections of normothermic rat neonates (Fig. 11) and hyperthermic rat newborns (Figs. 12-16) stained with haematoxylin and eosin ***Fig. (11)****: The normal structure of kidney showing the structure of renal corpuscle with its glomerulus (G) and Bowman's capsule (BW) (Fig. 11b X 512), the proximal (PX) and distal (DS) tubules and the collecting ducts (CT) (Fig. 11a X 128).* ***Fig. (12)****: Hyperplastic (H) glomeruli and swollen renal tubules (RT) with obliterated lumina seen in the hyperthermic rat newborns. (X 128)* ***Fig. (13)****: Necrosis in tubular cells and glomeruli (N) associated with the formation of albuminous material (AL) in the urinary space and the presence of some atrophied glomeruli (AG). (X 128)* ***Fig. (14)****: A photomicrograph illustrating fibroblasts proliferation (F) accompanied by necrosis (N). X 512)* ***Fig. (15)****: Sharply demarcated subcapsular area of necrosis (SN) seen after 2 hours of hyperthermia. (X 512)* ***Fig. (16)****: A photomicrograph showing edema (E) and inflammatory cells infiltration (IF). (X 512)*

sharply demarcated subcapsular areas of necrosis (Fig. 15) involving the renal tubules, glomeruli and even the walls of the blood vessels.

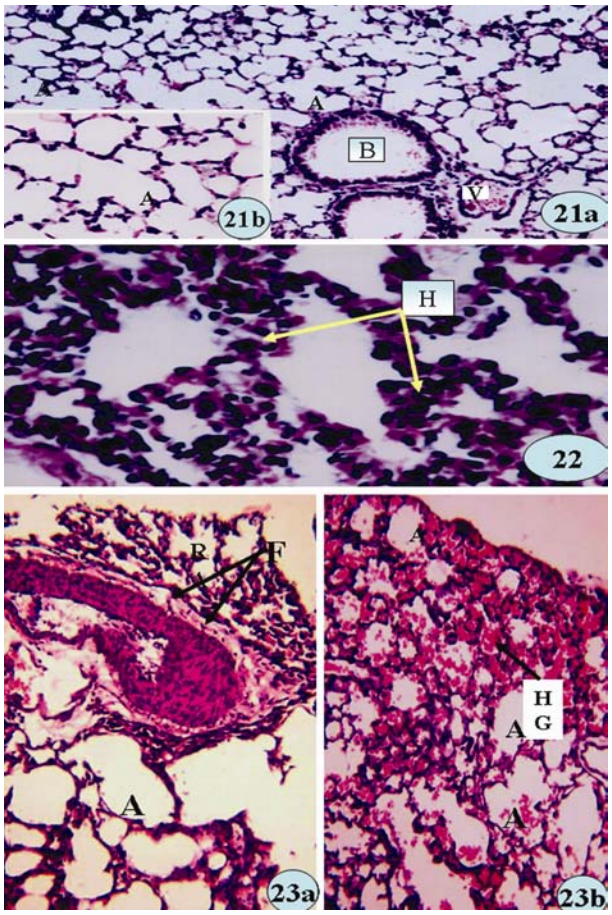
Masson's trichrome stain revealed low amounts of dispersed collagen around Malpigian corpuscles and renal tubules (Fig. 17) while it displayed marked amounts of collagen surrounding some Malpigian corpuscles (Fig. 18) and in between the renal tubules in a focal or diffuse manner (Figs. 19, 20).

The lung

The lung tissue of the control neonates showed normal structure of the alveoli (Fig. 21a & b) separated by intralveolar septa. Normally, an alveolus opens into respiratory bronchiole and is surrounded by perialveolar blood capillaries. The large bronchioles are lined with pseudostratified columnar ciliated epithelium (the mucosa). This mucosal layer is surrounded by connective tissue of the lamina propria, circular smooth muscle fibers and a thin layer of loose connective tissue (adventitia).

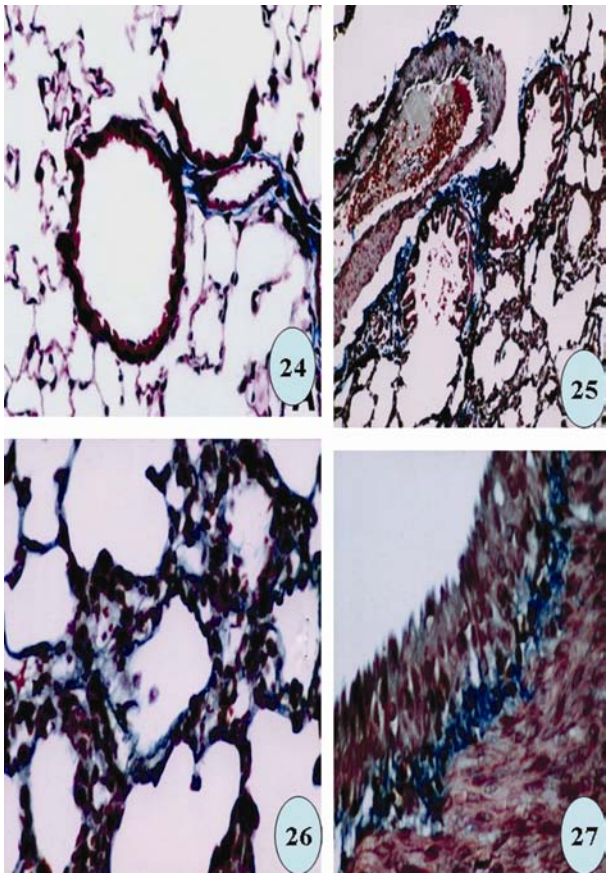


Figs. 17, 18, 19 & 20 represent collagen distribution (Blue green color) in the Masson's trichrome-stained kidney sections of normothermic rat newborns (Fig. 17) and hyperthermic ones (Figs. 18-20). (X512)



Figs. 21, 22 & 23 are representative photomicrograghs of lung sections of normothermic rat neonates (Fig. 21) and hyperthermic rat newborns (Figs. 22-23) stained with haematoxylin and eosin . ***Fig. (21)****: The normal lung structure illustrating the alveoli (A) lined with simple squamous epithelium (21b X 512), the bronchiole (B) with its columnar epithelial cells and the peribronchiolar blood vessel (V). (21a X 128)* ***Fig. (22)****: Hyperplasia (H) of the cells lining the alveolar walls of the lung of heat-exposed rat newborns. (X 512)* ***Fig. (23a)****: Emphysema of the alveoli (A) with rupture of some alveolar walls (RA) and peribronchiolar fibroblasts proliferation (F). (X 512)* ***Fig. (23b)****: Haemorrhage (HG) and emphysema of the alveoli (A) noticed in hyperthermic rat neonates. (X 512)*

In the lung of heat-treated neonates, hyperplasia of the cells lining alveoli (Fig. 22), alveolar haemorrhage, focal fibrosis and/or emphysema of the alveoli with rupture of some alveolar walls (Fig. 23a & b) were detected. The Masson's trichrome stain showed normal deposition of collagen fibers in the interstitial matrix of the walls of the alveoli and around the alveolar ducts and the openings of the



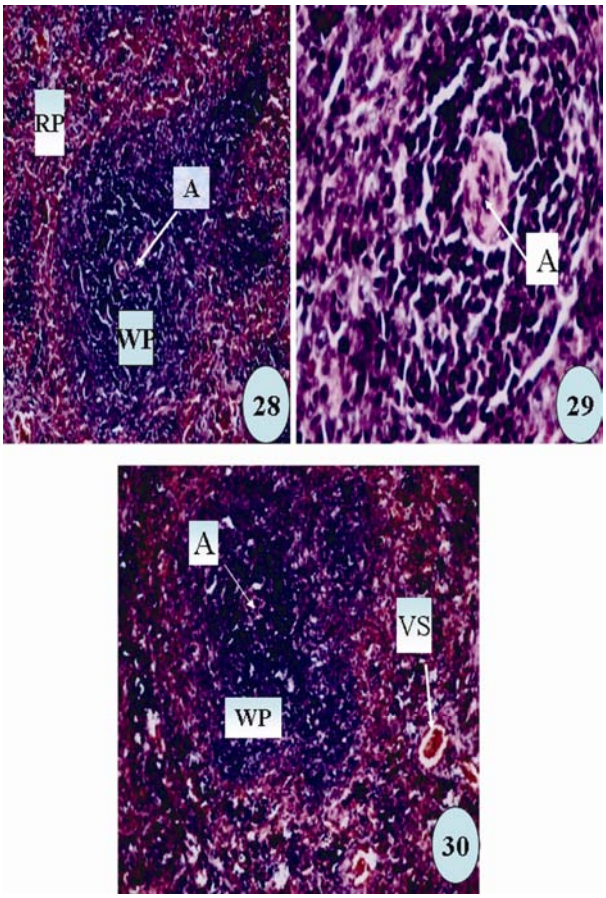
Figs. 24, 25, 26 & 27 represent collagen distribution (Blue green color) in the Masson's trichrome-stained lung sections of normothermic rat newborns (Fig. 24) and hyperthermic ones (Figs. 25-27). X 512 & 25 X 128

alveolar sacs and alveoli (Fig. 24). The collagen fibers around the alveolus merged with those around the openings to form a fine, three-dimensional supporting meshwork for the whole lung parenchyma. In heat-treated animals, the collagen was seen in the peri-bronchiolar (Fig. 25), interalveolar and perialveolar areas (Fig. 26) as well as in the tunica adventitia of the peribronchiolar dilated blood vessels (Fig. 27).

The spleen

Normally, the rat spleen consists of red pulps represented by blood sinuses filling the spaces between the white pulps which make the bulk of the organ highly vascular (Fig. 28) and white pulps represented by oval splenic discrete nodules of lymphoid aggregations surrounding the central arteries and forming what is known as periarteriolar sheath (Fig. 29).

The spleen of the treated group suffered from severe hyperemia in the red pulp and sinusoids with



Figs. 28, 29 & 30 are representative photomicrograghs of spleen sections of normothermic rat neonates (Figs. 28-29) and hyperthermic rat newborns (Fig. 30).stained with haematoxylin and eosin

***Fig. (28)**: Normal spleen architecture manifesting white pulps (WP), central arteries (A) and red pulps (RP). (X 128)*

***Fig. (29)**: higher magnification of the white pulp showing its nodular appearance of lymphoid aggregations around central arteries (A). (X 512)*

***Fig. (30)**: A photomicrograph showing severe hyperemia in the red pulps and sinusoids (VS) accompanied by distorted lymphoid nodules of the white pulp (WP). (X 512)*

distorted lymphoid nodules and atrophy of other parts (Fig. 30).

2- Quantitative analysis of fibrosis (Fig. 31):

Quantitative measurement of the degree of fibrosis proved a significant ($p < 0.01$) accumulation of collagen fibers in all the examined tissues except for the spleen.

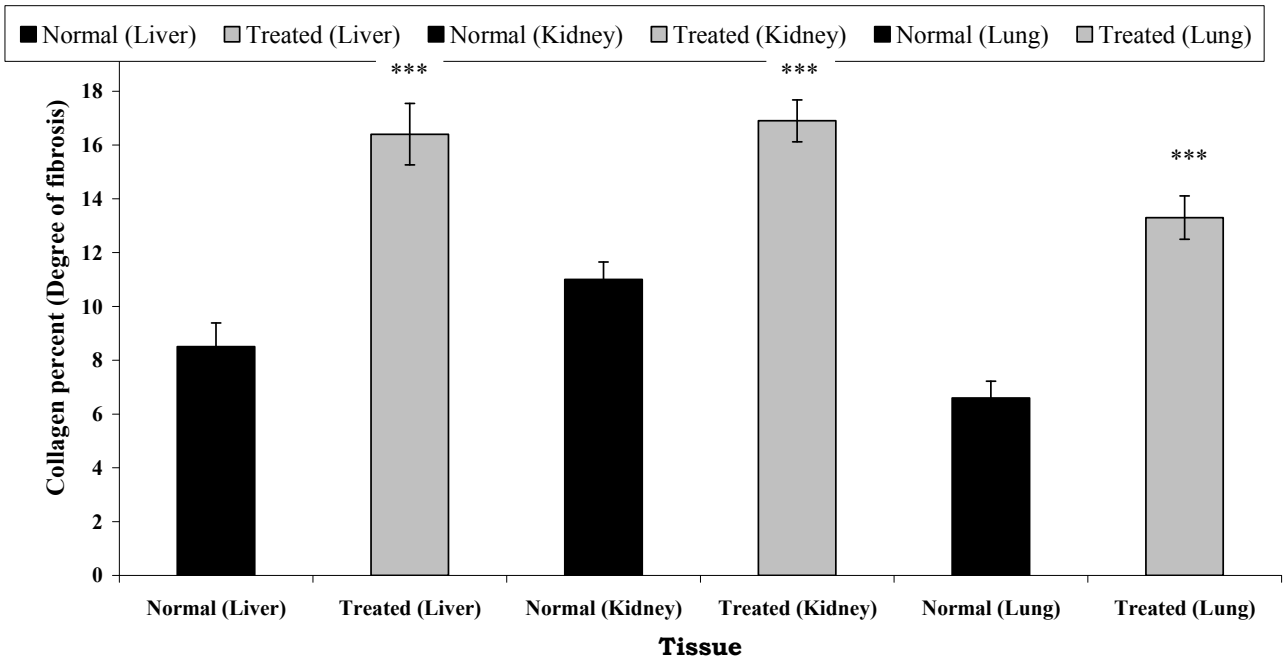


Figure 31: *Collagen Masson's trichrome percent*

3- Biochemical effects (Table 1)

The daily two hour exposure of rat offsprings to a high temperature (41°± 0.5°C) for 21 days produced a significant elevation of serum enzymes of the liver function, ALT, AST, GGT and LDH, and kidney function variables including urea and creatinine concentrations. Also, the concentration of serum total bilirubin, total protein and albumin were significantly increased while globulin content was significantly decreased. Uric acid concentration showed a non-significant elevation.

Discussion

Numerous clinical trials have applied hyperthermia in combination with radiation therapy and chemotherapy during the treatment of cancer [1,4, 41].

The liver

Liver and kidney temperature approximate whole body hyperthermia due to their extensive vascular

Table 1: *Effect of 21 days high temperature-exposure on various serum variables related to liver and kidney functions of rat newborn.*

Parameters	Groups	
	Normal	Treated
ALT activity (U/l)	34.600 ± 0.193	50.033 ± 0.365***
AST activity (U/l)	69.350 ± 0.290	84.550± 3.331**
GGT activity (U/l)	3.073 ± 0.055	4.507 ± 0.358***
LDH activity (U/dl)	51.500 ± 5.477	97.356 ± 9.174***
Total bilirubin concentration (mg/dl)	0.044 ± 0.001	0.216 ± 0.0483**
Total protein content (g/l)	48.300 ± 1.297	56.700 ± 0.134***
Albumin concentration (g/l)	24.700 ± 0.939	43.000 ± 0.365***
Globulin concentration (g/l)	24.100 ± 2.012	13.966 ± 0.388***
Creatinine content (mg/dl)	2.650 ± 0.022	3.000 ± 0.073**
Urea concentration (mg/dl)	36.550 ± 1.140	64.533 ± 1.7598***
Uric acid concentration (mg/l)	8.000 ± 2.236	12.500 ± 0.670

*Data are expressed as mean ± standard error. Number of animals in each group is six. * P< 0.0.5 significant, ** P< 0.01 highly significant, *** P< 0.001 very highly significant relative to corresponding normal values.*

network. The liver is a sentinel organ for thermal stress [42-43].

From the results of the present study on the effects of whole body hyperthermia on tested normal tissues, liver appeared to be at high risk of significant injury. The liver of hyperthermia-treated group showed distorted hepatic cords and fatty degenerative changes in the hepatocytes with proliferation of the bile ductules and infiltration of the parenchyma with inflammatory cells. This infiltration may be accompanied by necrosis of the parenchyma mainly around the portal area. Using Masson's trichrome stain showed massive deposition of collagen fibers in between the hepatocytes forming pseudolobules (cirrhotic change).

Similar but less striking liver changes like vacuolization, focal necrosis, haemorrhage, focal fibrosis, vessel rupture, small vessel dilatation, generalized sinusoid dilatation, proliferation of bile duct and damage of liver lobules have been described after treatment with hyperthermia [43-49].

On the contrary, Hotchkiss et al. [50], Kluger et al. [51] and Suganuma et al. [18] reported anti-inflammatory actions of whole body hyperthermia in different organs of rodents.

The present-noticed necrotic pattern as the result of hyperthermia could be induced by ischemia resulting from the sluggish capillary blood flow that may also be the cause of vessel rupture and haemorrhage [2-3, 44, 52] or it may be the result of endotoxaemia that drives hyperthermia and triggers systemic coagulation, haemorrhage, necrosis, cell death and multiorgan failure [53].

Hepatic fibrogenesis is often associated with hepatocellular necrosis and inflammation [54-55]. The hallmark of fibrosis is collagen deposition [56] since the inflammatory response includes migration and activation of both resident and circulating inflammatory cells and the production of cytokines and growth factors. Activated inflammatory cells and released cytokines stimulate multiplication, migration, secretory activities and collagen production by fibroblasts. Hepatic fibrosis is a common response to chronic liver injury of variable origins which can lead to cirrhosis and ultimately end stage liver failure and increased risk for hepatocellular carcinoma [57-59].

Biochemically, the results of serum liver function tests of rat newborn subjected to whole body hyperthermia for two hours along 21 days showed elevation of ALT, AST, GGT, LDH, total bilirubin, total protein and albumin measures and a decrease in globulin concentration value if compared to control.

In agreement with these results, interstitial hyperthermia applied to livers of dogs determined a rise in AST, ALT and LDH [46]. Also, hyperthermia applied to patients suffering from cancer showed increased LDH and AST values [19]. Similarly, biochemical assays of blood of cancer patients during intraperitoneal chemohyperthermia revealed increased levels of ALT, AST, and bilirubin and a decrease in the level of GGT [21].

Contrary to our results, Streffer [20] reported an immediate decrease of protein synthesis as the result of hyperthermia in embryos of mice.

Serum enzymes elevation after hyperthyermia may indicate the hepatocellular injury [60,61] or a compensatory action of the body [61].

The elevated total bilirubin in serum may reflect necrosis of hepatic parenchyma that seems to move conjugated bilirubin from necrotic hepatocytes to the sinusoids or the result of bilirubin drainage blocking due to inflammatory cells infiltration in portal areas [62].

On the other hand, the increase in the total protein content in the sera of hyperthermic rat neonates may be attributed to the diminished capability of liver to carry out gluconeogenesis as a result of thermal stress [63], decreased rate of amino acid conversion to glucose or an increased protein synthesis secondary to increased amount and availability of mRNA.

The kidney

Because of its high blood flow rate, the renal parenchyma has not been considered by some investigators as a likely site of hyperthermia injury. Some studies, however, suggest that this is not the case [56].

In the present study, kidney of heat-exposed rat neonates showed glomerular, tubular and even vascular areas of necrosis associated with edema and infiltration of inflammatory cells. However, some glomeruli became hyperplastic. Masson's trichrome stained kidney sections displayed marked amounts of collagen surrounding some Malpigian corpuscles and in between the renal tubules in a focal or diffuse fashion.

Consistent with our results, Elkon et al. [6] reported damage in the proximal tubules of the subcapsular region and necrosis of tubules or glomeruli in a circumscribed area in the mouse kidney as a response to exposure to a range of hyperthermic temperatures (41°- 45°C). Moreover, Mella et al. [64] reported only proximal tubular necrosis in BD IX rats.

The present histopathological changes of kidney were associated with a significant elevation of serum urea, uric acid and creatinine concentrations.

The elevated serum levels of kidney function variables after hyperthermia have also been noticed by many authors [6, 19, 21, 65]. The increase in the renal biochemical variables may reflect the existence of a significant degree of cellular lysis. Additionally, hyperthermia-related increase in serum creatinine, urea and uric acid are indicative values reflecting not only kidney function impairment [66-67], but also endpoints that detect treatment-related toxic effects of any treatment in the kidney of rats [68].

The lung

Hyperthermia in the present study induced a variety of histopathological changes in lung mainly including hemorrhage, emphysema of the alveoli, hyperplasia of the cells lining alveoli and focal fibrosis. Moreover, the peribronchiolar dilated blood vessels, and the interalveolar and perialveolar areas showed greater amounts of collagen as revealed by Masson's trichrome stain.

In agreement with the present results, Baciewicz et al. [15] reported vascular disruption with severe widening of the pulmonary interstitium and severe haemorrhage. Also, Suzuki et al. [17] recorded edema and alveolar haemorrhage in the lungs of hyperthermic-treated rabbits.

The increased serum protein concentration suggests that high temperature may disturb alveolar capillary barrier of lung by causing different degrees of injury that increase the permeability of the pulmonary epithelium and endothelium, resulting in extravasation of plasma proteins and ultimately extracellular matrix remodelling and collagen deposition in the alveolar lumen and interstitium. Moreover, the increased LDH value may represent the extent of damage to lung cells. The inflammatory response is the initial response following the injury and fibrosis is then generally a final outcome.

The spleen

Histopathological examination of the spleen of hyperthermic rat neonates revealed severe hyperemia in the red pulps and sinusoids with distorted lymphoid nodules and atrophy of the other parts.

Solov'ev [69] reported enhanced lymphocyte function, proliferative responses, and suppressed T-cell immune response in spleen after acute hyperthermia. Moreover, Timoshenko and Cherenkeviche [70] found that hyperthermia decreased the rate of rat thymocytes as well as solenocytes aggregation via shedding of plasma membrane glycoproteins and changes of surface charge.

The present-noticed negative inflammatory response of the spleen of hyperthermic rat neonates may be an indication of decreased levels of IL-1 produced from phagocytic lining cells of the spleen where it enters the circulation and acts as a hormone to mediate responses to infection, immunologic reaction and inflammatory process.

Conclusion

Despite the promising results and an increased response of malignant tumours to radiation and/or chemotherapy after hyperthermia and the assumption that the high preferential absorption ability of tumour tissue components to heat over normal tissues rely, in part, upon their higher collagen and protein content, attention should be paid to the repetitive effect of high temperature on normal tissues architectures , total collagen and protein contents in addition to its upshots on other various biochemical indices to appraise the impact of the method on patients, particularly newborns, and those with recurrent carcinoma.

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