THE CHANGE OF HEAT SHOCK PROTEIN AND TESTOSTERONE DURING THE RECOVERY FROM MILD AND SEVERE HYPERTHERMIA IN MALE RABBITS.

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ABSTRACT: Hyperthermia therapy, a form of cancer treatment, has been recently paid considerable attention because it is expected to significantly reduce clinical side effects compared to chemotherapy and radiotherapy and can be effectively used for killing localized or deeply seated cancer tumors. The present study was designed to investigate the thermal kinetics of mild and severe whole body hyperthermia (WBH) at 42°C and 43°C and its thermal late effect, at 7, 14, 21 and 28 days post mild and severe WBH (42°C & 43°C) in normal strain male rabbits, on some biochemical parameters, serum levels of heat shock proteins, total protein, albumin, globulin, and testosterone. Besides the histological structure of testis, before and after mild and severe WBH treatment and its thermal late effects at 7, 14, 21 and 28 days. It was attempted to evaluate the degree of safety and the effective time when hyperthermia is applied in the clinic for treating cancer and other diseases with keeping the normal cell intact. Exposure of male rabbits to mild (42°C) and severe (43°C) WBH showed an increase in serum total protein and albumin which showed continuous increase till the end of experiment in mild WBH (42°C) whereas it induced significant decrease in serum globulins. While in severe treatment (43°C), serum total protein showed significant decrease and reached the control value at 28 days post heat treatment. Severe WBH (43°C) showed significant increase in serum albumin and globulins immediately after WBH till the end of the experiment (from 7 days till 28 days). Results showed that significant decrease in testosterone level immediately after mild (42°C) and severe (43°C) WBH. During the recovery period the level of testosterone began to increase till reached nearly the control value at 28 days post WBH in both treatments (42°C and 43°C).Moreover, mild & severe WBH (42°C&43°C) caused induction of HSP70 KD of rabbit serum immediately after 7, 14, 21 and 28 days. The histopathological examination of testis revealed that the mild treatment group (at 42°C) the duration time was long through the period of the experiment (28 days) with mild changes in the testicular tissues which characterized by mild degeneration, azosperms and giant spermatogonial cell formation. Regarding the process of spermatogenesis in this group, it is still weak through the whole period of the experiment (28 days) but it did not stop. In the severe treatment group (43°C), there was severe reaction characterized by degenerative, necrobiotic changes in the early stage and immediately after heat exposure with short duration time until 14 days post WBH, while at 21 days and 28 days, the tissue started to show completely series of spermatogenesis and mature sperms associated with giant spermatogonial cells at 28 days post WBH (43°C). The spermatogenesis was completely affected and nearly stopped then went back to normal as quickly as 21 days post WBH (43°C). [New York Science Journal 2010; 3(6):74-87]. (ISSN 1554 – 0200).

Keywords: Hyperthermia therapy; histopathological examination; mild degeneration; azosperms

1. INTRODUCTION:

Hyperthermia is deliberate heating of the whole body to achieve an elevated core temperature for therapeutic purposes. Other terms used are whole-body hyperthermia, systemic or whole body thermal therapy, and hyperpyrexia. Typically, core body temperatures of 41–42 ◦C are induced for 1–2 h, or alternatively 39–40 ◦C for 4–8 h. **(Rowe, 2007).** Therefore, hyperthermia has been used for many years to treat a variety of malignant tumors **(Saito *et al*., 2007**).

Research has shown that high temperatures can damage and kill cancer cells with usually minimal injury to normal tissues **(Vanderzee, 2002).** Killing of cancer cells results from damaging proteins and structures within cells **(Hildebrandt et al., 2002)**.

Hyperthermia induced heat-shock proteins that provide a danger signal to the immune system. This danger signal recognized by naturalkiller cells, can awaken the immune system to fight back **(Falk and Issels, 2001 and Hu et al., 2008)**.

The time required for the core temperature to approach its steady-state value allowed sufficient time for the tissues to synthesize heat shock proteins (HSPs), which are known to provide some protection against thermal damage, including that induced by fever (**Jones et al.,2003 and Giombini et al.,2007)**.

Heat shock proteins (HSPs) are synthesized by cells of all organisms as resistance to internal and external cellular stressors including physical stress, metabolic stress and diseases. Temperature resistance is dependent on the ability of HSPs to function as molecular chaperones and prevent aggregation and on the capacity of Hsp27 and Hsp70 to act as wide

spectrum inhibitors of the cell death pathways. **(Calderwood and Ciocca 2008** and **Bolhassani and Rafati, 2008)**.HSP70 is well known for being the most responsive to heat stress and exercise. Hsp70 proteins are central components of the cellular network of molecular chaperones and folding catalysts. They assist a large variety of protein folding processes in the cell by transient association of their substrate binding domain with short hydrophobic peptide segments within their substrate proteins. The substrate binding and release cycle is driven by the switching of Hsp70 between the low-affinity ATP bound state and the high-affinity ADP bound state. Thus, ATP binding and hydrolysis are essential in vitro and in vivo for the chaperone activity of Hsp70 proteins. (**Mayer and Bukau 2005).**

MATERIALS AND METHODS

The present study was carried out in the Radiobiological Chemistry Unit, Radioisotopes Department, Nuclear Research Center, Atomic Energy Authority, Cairo, Egypt.

a) Animal:

Normal 38 male adult local strain rabbits were breed in Radioisotopes Department, Atomic Energy Authority.

Rabbits were chosen as a model for this study because rabbits can withstand repeated total body hyperthermia at 42°C & 43°C. In addition, rabbits readily tolerate repeated blood sampling and the procedures are technically easy **(Shah and Dickson, 1978; Favarato and Zatta 1990 and Mostafa et al., 2002 & 2007 a&b)**.

b) Experimental Design:

38 normal adult local strain male rabbits at 12 months old and 1500 $\pm$ 200g body weight (BW) were used in the present studies, 12 animals were used for 42°C whole body hyperthermia (WBH) treatment and 16 animals for 43°C (WBH) treatment. 10 animals were used for histological studies.

Every two rabbits were transferred from their cages to temperature-controlled heating box for WBH (42°C & 43°C) treatments after having their meals and drinking fresh water.

The ambient temperature and relative humidity (RH) were set at 45-47°C and 18-23%. Rectal temperature (RT) of rabbits was recorded using a thermocouple (cole-parmer type T thermocouple thermometer).

During each of the WBH treatments (42°C & 43°C), the RT was recorded at 15 minutes intervals, the heat exposure was terminated either after 1.5 hr or when RT reached just below the (42°C & 43°C), which ever reached first. Directly after WBH treatments, the animal was weighed again and a blood sample (1.5 ml) was collected. The animals were allowed to be recovered at room temperature. The blood samples were taken at 7, 14, 21 and 28 days following each WBH treatments (42°C & 43°C). The blood samples were allowed to clot, centrifuge and separated serum samples were stored at -20°C until assayed for testosterone by a solid-phase radio-immunoassay **(Jaffe and Behrman, 1974; Demetriou, 1987 and Wilson and Foster, 1992)**. Using a kit total serum protein was used according to method of **(Weischelbaum (1946) and Gornal et al. (1949)**, albumin **Doumas et al. (1971)**, and electrophoresis of protein was carried out using one dimensional polyacrylamide **(Laemmli, 1970)**.Beside the testis tissues were removed from animals immediately after the experiment termination, at recovery period 7, 14, 21 and 28 days post WBH treatments at 42°C & 43°C. The dissected samples of testis, liver, kidney and heart were placed in 10% neutral buffered formation for few days, dehydrated in ascending concentrations series of ethyl alcohol (70-100%) and then prepared using standard procedures for hematoxylin and eosin staining as described by **Humason (1972)**.

Statistical analysis:

Student's t' test was used to evaluate the data collected for the effect of whole body hyperthermia on the various biological and physiological parameters investigated this study.

RESULTS:

Data presented in table (1) reveal that mild WBH at 42°C induced a sudden increase in total serum protein level from 5.7 $\pm$ 0.07 to 6.1 $\pm$ 0.06 g/dl which was significant ($P < 0.001$) compared to the control. This increase amounted to 7.4%.Severe WBH at 43°C showed significantly ($P < 0.001$) higher increase in total serum protein in comparison with the mild heat exposure (42°C). This increase was 13.4% higher than the control. The total serum protein showed continuous increase the till end of experiment (28 days) in mild heat treatment at (42°C). While, in severe treatment at

(43°C) the total serum protein level showed significant decrease and resembled the control value at the 28 days of treatment (Table 2).

Table (1) showed that mild WBH at 42°C induced significant increase in serum albumin from 3.9 ± 0.1 to 4.4 ± 0.1 g/dl which was significant (P<0.001) compared to the control. This increase amounted to 10.3%. Severe WBH at 43°C showed a significant increase (P<0.001) from 3.9±0.1 to 4.8±0.1 which amounted to 21.1%. Afterwards the serum albumin showed continuous increase in both treatments till the end of experiment (28 days) approaching the control value. The correlation between serum albumin values and the time of recovery was significant in both treatments. It was evident that mild WBH at 42°C induced a significant decrease (P < 0.001) in serum globulin level from 1.9 ± 0.1 to 1.6 ± 0.04 g/dl above the control. This decrease amounted to 28.8%. Severe WBH at 43°C showed a significant increase (P<0.001) in serum globulin level from 1.9 ± 0.1 to 2.4 ± 0.04 g/dl. This increase amounted 28.8% higher than the control. Afterwards the serum globulin showed significant increase throughout the experiment (28 days) of both treatments (42°C & 43°C).

Table (1): Effect of whole Body Hyperthermia (WBH) at 42°C & 43°C and its late effects on total serum protein, albumin, globulin and testosterone of male rabbits.

Parameters	Temperature	Before (WBH) control	Immediately after (WBH)	Late effect (Recovery)			
				7 days	14 days	21 days	28 days
Total serum protein (g/dl)	42(°C)	5.7±0.07	6.1±0.06***	6.06±0.13**	6.1±0.12**	6.4±0.15***	6.5±0.16***
	% of change		7.4%	5.9%	6.3%	3.3%	13.3%
	43.(°C)	5.7±0.09	7.1±0.06***	6.3±0.12***	6.1±0.1**	5.8±0.11*	5.7±0.08
	% of change		13.4%	9.8%	6.0%	0.87%	-
Albumin (g/dl)	42(°C)	3.9±0.1	4.4±0.1***	4.2±0.1***	4.06±0.03*	3.96±0.1	4.0±0.1*
	% of change		10.3%	5.9%	3.2%	0.43%	2.1%
	43(°C)	3.9±0.1	4.8±0.1***	4.5±0.04***	4.3±0.1**	4.1±0.1**	4.0±0.1*
	% of change		21.1%	13.6%	7.87%	3.4%	2.0%
Globulin (g/dl)	42(°C)	1.9±0.1	1.6±0.04***	2.3±0.03***	2.0±0.1*	2.2±0.1***	2.3±0.04***
	% of change		-14.2%	23.6%	5.8%	15.2%	21.0%
	43(°C)	1.9±0.1	2.4±0.04***	2.0±0.08	1.6±0.05***	2.1±0.1***	2.0±0.1***
	% of change		28.8%	5.4%	-11.6%	14.7%	7.1%
Testosterone (mg/dl)	42(°C)	3.5±0.1	3.1±0.1***	3.07±0.1***	3.6±0.1***	3.2±0.1**	3.5±0.1*
	% of change		-11.9%	-11.6%	14.1%	-7.6%	-0.5%
	43(°C)	3.5±0.1	3.0±0.1***	3.2±0.05**	3.0±0.1*	3.3±0.1*	3.5±0.1
	% of change		-12.1%	-7.8%	-14.1%	-4.6%	-0.2%

All values are expressed as Mean ± SE "n" for 42°C = 12 animals

*** Significant at P < 0.001 ** Significant at P < 0.01 * Significant at P < 0.05 "n" for 43°C = 16 animals

Table (2): Statistical Differences between 42°C & 43°C means of parameters at different times after heat exposure.

Parameters	Immediately after (WBH)	Late effect (Recovery			
		7 days	14 days	21 days	28 days
Total serum protein	-10.398***	-1.378	-0.147	2.67*	4.0***
Albumin	-4.0***	-3.923***	-2.044	-1.258	0.516
Globulin	13.66***	3.908***	3.611**	0.088	2.40*
Testosterone	0.268	1.1	0.49	5.495***	0.55

*** Significant at P < 0.001 ** Significant at P < 0.01 *Significant at P < 0.05

Table (3): Effect of whole body hyperthermia (WBH) at 42°C & 43°C and its late effects on heat shock proteins "HSPs" of male rabbits

Time of heat exposure	42°C			43°C		
	Mean ± SE	% of change	"t" value	Mean ± SE	% of change	"t" value
Before WBH (control)	8.58±0.4	-	-	8.697±0.37	-	-
Imm. After WBH	16.02±1.03	86.71%	7.9**	12.5±1.7	43.7%	2.1
7 days post WBH	10.78±2.6	25.6%	0.83	5.21±1.5	-40.1%	1.56
14 days post WBH	8.05±1.02	6.2%	0.22	12.46±1.7	43.3%	2.47
21 days post WBH	5.27±2.4	38.5%	4.24*	12.36±1.04	42.1%	2.21
28 days post WBH	7.26±2.7	15.3%	1.91	12.599±1.4	44.8%	1.84

All values are expressed as Mean ± SE n (number of animals) = 3 * Significant at $P < 0.05$ ** Highly significant at $P < 0.01$

Table (1) showed that mild WBH at 42°C induced significant decrease ($P<0.001$) in the level of testosterone from 3.5 ± 0.1 to 3.1 ± 0.1 mg/dl and this decrease was amounted to -11.9%. Also severe WBH at 43°C induced a significant decrease from 3.5 ± 0.1 to 3.0 ± 0.1 mg/dl which amounted to -12.1% below the control value. Afterwards the level of testosterone begin to increase till reached nearly to the control value at 28 days post WBH in both treatments (42°C & 43°C).. The level of testosterone showed a significant difference ($P<0.001$) only at 21 days post heat treatment between 42°C and 43°C WBH (Table 2).

Table (3) and Fig. (1a) revealed that mild (WBH) at 42°C induced a sudden increase in HSPs amount from 8.58 ± 0.4 to 16.02 ± 1.03 which was significant ($P<0.01$) above the control. This increase amounted to 86.71%. Severe WBH at 43°C (Fig 1b) showed an increase in the amount of HSPs from 8.697 ± 0.37 to 12.5 ± 1.7 and this increase was 43.7% compared to the control. Afterwards HSPs values at mild WBH (42°C) showed a continuous decrease at 7 days (but above the control) and showed a significant decrease at 21 days till the end of experiment 28 days post WBH. While at severe WBH (43°C) the HSPs values decreased at 7 days post WBH but increased afterwards at 14, 21 and 28 days post WBH (Table 3).

Histological studies of the testis:

Control testis: As shown in (Fig. 1).

Testis of rabbit exposed immediately after mild WBH (42°C) showed severe dilatation and hyperemia in the stromal blood vessels with absence of sperms from the lumenae of the seminiferous tubules (Fig. 2).

Severe WBH (43°C) showed degenerative and necrobiotic changes in the spermatocytes of the seminiferous tubules with appearance of pyknotic nuclei as well as fragmentation in the nuclear material of some spermatogonial cells in association with complete azosperm in the tubular lumen and hyperemia in stromal blood vessels and capillaries (Fig. 3).

At 7 days post mild WBH (42°C) showed hyperemia was noticed in the stromal blood vessels and capillaries, while the seminiferous tubules showed degenerative change with appearance of giant spermatogonial cells in the tubular lumenae. There was hyalinization in the cytoplasm and pyknosis in the nuclei of the giant spermatogonial cells in some seminiferous tubules (Fig. 4). While, 7-days post severe WBH (43°C) showed severe degenerative and necrobiotic changes were observed in the spermatocytes of the seminiferous tubules with complete azo sperm in the tubular lumen and hyperemic stromal blood vessels and capillaries (Fig. 5).

Testis of rabbit 14 days post mild WBH (42°C) showed giant spermatogonial cells in the lumenae of some seminiferous tubules (Fig.6). While 14-days post severe WBH (43°C) showed severe degeneration in the seminiferous tubules with

complete azosperm in the tubular luman and appearance of one cells layer of the spermatogonial cells adjacent the basal cell layer beside the hyperemia in the stromal vascularity (Fig. 7).

21-days post mild WBH (42°C) showed multiple numbers of giant spermatogonial cells were detected in the lumenae of most of the seminiferous tubules (Fig. 8). While 21-days post severe WBH (43°C) showed complete series of spermatogenesis in the active seminiferous tubules with appearance of mature sperms in the central portion of the lumen (Fig. 9).

Testis of rabbit after 28-days post mild WBH (42°C) showed multiple number of giant spermatogonial cells with hyalinized cytoplasm and pyknotic nuclei in the seminiferous tubular lumen (Fig. 10).As well as, 28-days post severe WBH (43°C) showed complete series of spermatogenesis with giant spermatogonial cells and mature sperms in the lumen of seminiferous tubules (Fig. 11).

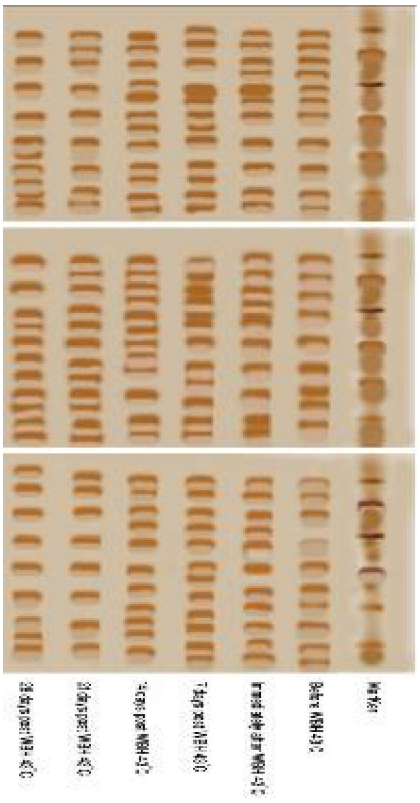


Fig (1a)

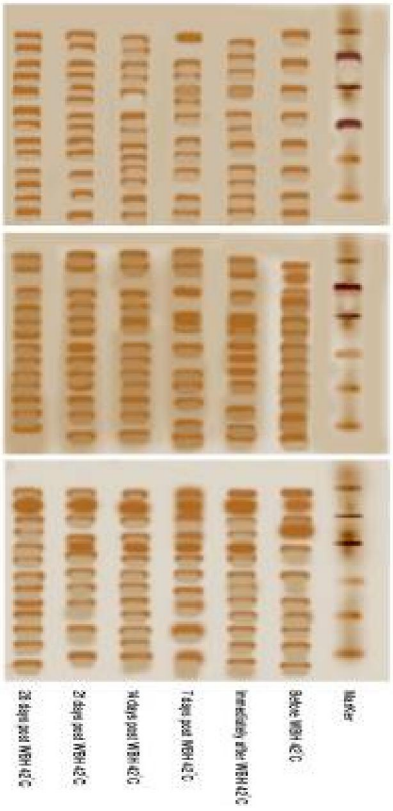
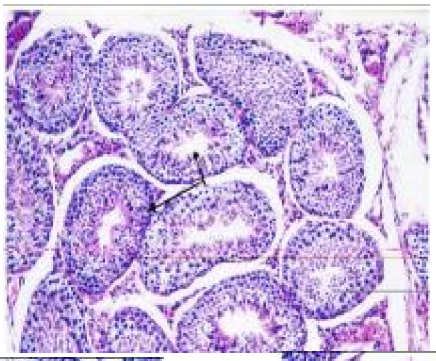


Fig (1b)

Fig. (1a&b): Effect of whole body hyperthermia (WBH) at 42°C & 43°C and its late effects on heat shock proteins "HSPs" of male rabbits



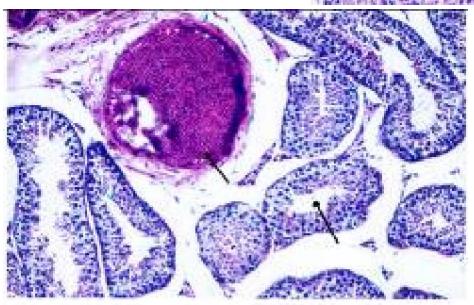


Fig (2)

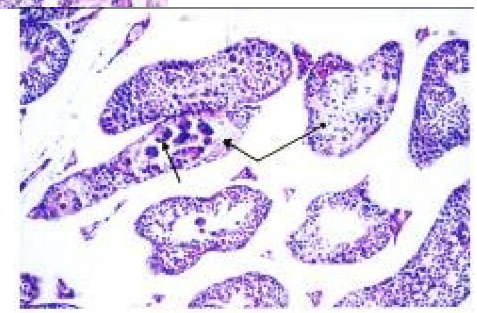


Fig.(4)

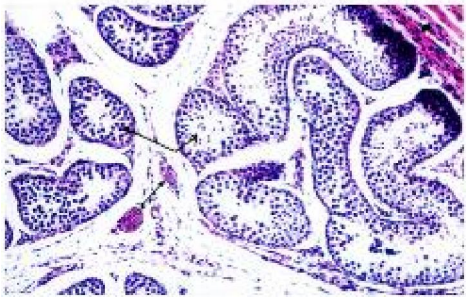


Fig.(3)

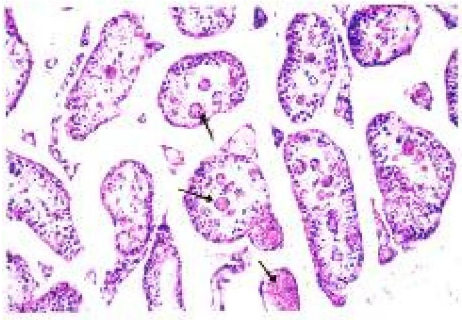


Fig (5)

Fig. (1): Section in testis of a control rabbit showing the normal histological structure of the mature seminiferous tubules with complete series of spermatogenesis (arrows)

Fig. (2):Section in testis of rabbit immediately after mild WBH (42°C) showing severe dilatation and hyperemia in the interstitial blood vessels with absence of the sperms from the lumen of seminiferous tubules (arrows)

Fig. (3): Section in testis of rabbit immediately after severe WBH (43°C) showing degenerated seminiferous tubules and agosperm with hyperemic blood capillaries (arrows).

Fig. (4): Section in testis of rabbit 7 days-post mild WBH (42°C) showing degeneration in some individual seminiferous tubules with appearance of giant spermatogonial cells in the tubular lumenae (arrows)

Fig. (5): Section in testis of rabbit 7 days-post severe WBH (43°C) showing severe degenerative and necrobiotic changes in the spermatogonial cells of the seminiferous tubules with dilated stromal blood vessels and capillaries associated with azospermia (arrows).

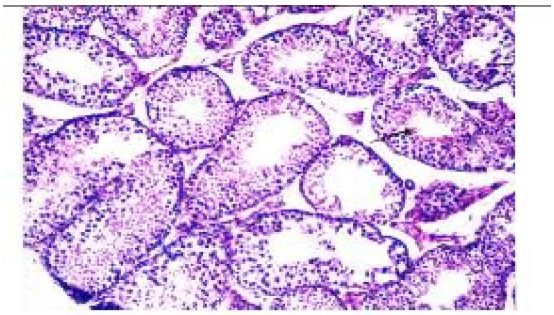


Fig (6)

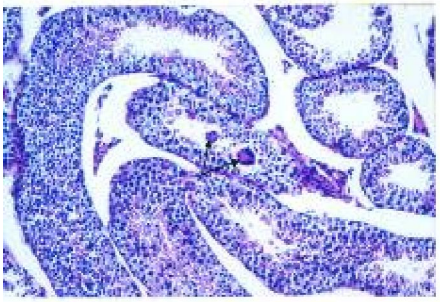


Fig (8)

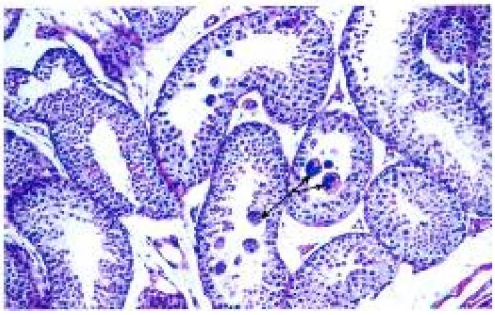


Fig (7)

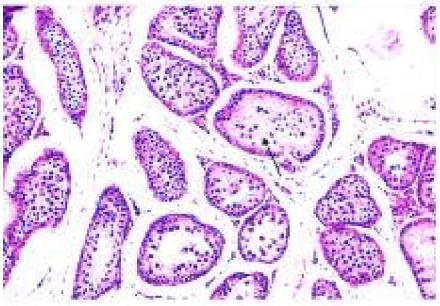


Fig (9)

Fig. (6): Section in testis of rabbit 14 days-post mild WBH (42°C) showing giant spermatogonial cells formation in the lumen of seminiferous tubules (arrows).

Fig. (7): Section in testis of rabbit 14 days-post severe WBH (43°C) showing severe degeneration in the spermatogonial cells in the seminiferous tubules associated with azospermia (arrows).

Fig. (8): Section in testis of rabbit 21 days-post mild WBH (42°C) showing multiple number of giant spermatogonial cells in the lumen of seminiferous tubules (arrows).

Fig. (9): Section in testis of rabbit 21 days-post severe WBH (43°C) showing complete series of spermatogenesis in the mature seminiferous tubules with sperms in the central portion of the lumenae (arrows).

(H x & E. x 40)

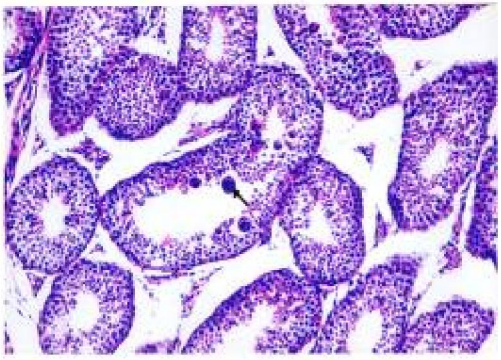


Fig (10)

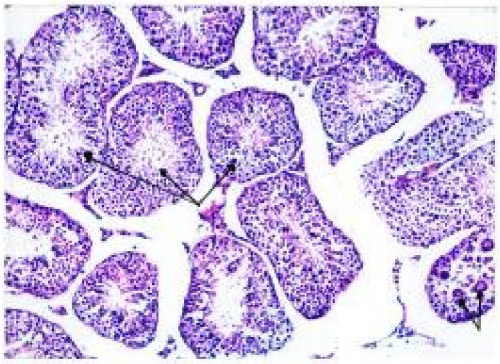


Fig (11)

Fig. (10): Section in testis of rabbit 28 days-post mild WBH (42°C) showing multiple number of giant spermatogonial cells in the lumen of the degenerative seminiferous tubules (arrows). (H x & E. x 40).

Fig. (11): Section in testis of rabbit 28 days-post severe WBH (43°C) showing complete series of spermatogenesis in the seminiferous tubules of mature testicular tissue with giant spermatocytes formation (arrows) in some individual lumenae (arrows).

(H x & E. x 40).

DISCUSSION

The mild and severe WBH (42°C & 43°C) induced an increase in total serum protein of all rabbits used in this study. This result is in agreement with previous studies **(Marder et al., 1990; Chiericato et al., 1994 and Mostafa et al., 2002 & 2007 a,b)**. Total serum protein is generally elevated due to dehydration caused by heat shock and hyperthermia **(Fox, 1989 and Marder et al. 1990)**. It may be also attributed to the heat-induced impairment in the cellular membrane permeability **(Koter, 1992)**.

Mostafa et al. (2007a) reported significant increase in total serum protein under the effect of WBH (43°C). During recovery period (24 hr post WBH), the total serum protein decreased than the acute WBH (43°C). **Mostafa et al. (2007b)** found that WBH treatment (42.5°C) of female rabbits caused a significant increase in total serum protein. That increase occurred immediately after exposure to WBH (42.5°C) and it continued after 1,2,4 hrs post WBH (42.5°C) and decreased at 8 till 24 hr post WBH.

In the present study, total serum protein showed continuous increase till the end of experiment in mild WBH (42°C). While in severe treatment (43°C), serum total protein showed significant decrease and reached the control value at 28 days post heat treatment. This finding indicates that the

changes induced by severe WBH are partially reversible. While mild WBH (42°C) induced significant increase in serum albumin, it caused a significant decrease in serum globulin. Severe WBH (43°C) showed a significant increase in serum albumin and globulin immediately after WBH. Afterwards the serum albumin and globulin showed a significant increase in both treatments till the end of the experiment (from 7 days till 28 days).

In the present study the significant increase in serum albumin and globulin was correlated with the significant increase in serum total protein. This is in accordance with data of **Marder et al. (1990)** who reported elevation of total protein and globulin after hyperthermia of rabbits.

Testosterone is the critical hormone that maintains spermatogenesis in the testis **(Kasahara et al., 2002)**. The testis is sensitive to a variety of stressors, such as hyperthermia, inflammation, radiation and exposure to agents that induce apoptosis of germ cells **(Hasegawa et al., 1997 and Jia et al., 2008)**. Heat causes a rapid and transient suppression of spermatogenesis' **(Wang et al., 2007 and Shefi et al., 2007).**

The environmental temperature can influence notably the hormonal profile of the rabbit by conditioning the productive performance of the animals **(Chiericato et al., 1995).** It was observed that high temperature levels significantly altered the performance of growing rabbit and their plasma androgen and thyroid profiles **(Botti et al., 1992)** and also, altered the hormonal pattern **(Marai et al., 1990 and Habeeb et al., 1993)**.

The present study showed significant decrease of testosterone hormone immediately after mild WBH (42°C) and severe WBH (43°C). Such a decrease is thought to be related to the physiological activity of Sertoli cells under the control of FSH and LH that has a major effect on the biosynthesis of testosterone **Contreras,(2006))**. This is in agreement with that of **El Masry et al., 1994** who studied the influence of hot summer (35° $\pm$ 3°C and 46 $\pm$ 8% RH) on New Zealand white (NZW) rabbit males (12-15 month old) they reported significant decrease in testosterone hormone. It could be noted that the decline in sperm concentration is parallel with the level of testosterone in blood. This is also, in accordance with **Chiericato et al. (1995)** who reported that, the significant decrease ($P < 0.05$) for testosterone (T), dihydrotestosterone (DHT) and T + DHT plasma concentrations agree with previous findings using other genotypes of rabbits **(Moor and Younglay, 1975; Chiericato, 1984 and Botti et al., 1992)**.

During the recovery period, the level of testosterone began to increase till reached nearly to the control value at 28 days post WBH at both treatments (42°C & 43°C). Heat shock proteins (HSPs) are required for spermatogenesis and also protect cells from environmental hazards such as, heat, radiation and chemicals. Cellular and molecular methods were used to characterize effects of testicular heat shock (43°C for 20 min) at different times post treatment **(Rockett et al., 2001)**. Based on histopathology, spermatocytes are the most susceptible cell type. Apoptosis in spermatocytes was confirmed by TUNEL, and was temporally correlated with the expression of stress-inducible HSP70-1 and HSP70-3 proteins in spermatocytes **(Rockett et al., 2001)**.

Because oxidative stress on the testis is one of the major factors that induce germ cell apoptosis, this organ has fairly high concentrations of anti-oxidants, such as GSH, ascorbic acid and vitamin E **(Rao and Shata 2000 and Yang et al., 2001)**. These antioxidants protect germ cells against oxidative DNA damage and play an important role in spermatogenesis **(Kasahara et al., 2002)**.

The present data were confirmed by the histological sections of testis of male rabbit immediately after WBH (42°C & 43°C) and during late effects (recovery period, 28 days post WBH) in mild treatment (42°C) and severe treatment (43°C).

Heat shock proteins (HSPs) could play an important physiological role in normal cell functioning. Under normal conditions, heat-shock proteins play numerous roles in cell function, including modulating protein activity by changing protein conformation, promoting multiprotein complex assembly / disassembly, regulating protein degradation within the proteosome pathway, facilitating protein translocation across organellar membranes, and ensuring proper folding of nascent polypeptide chains during protein translation **(Takayama et al., 2003)**.

Heat shock proteins are produced in response to different types of stress conditions making cell resistance to stress-induced cell damage **(Takayama et al., 2003 and Madden et al., 2008)**. Moreover, these stress proteins seem to be expressed by some cells living in physiological condition.

HSPs are evolutionary conserved polypeptides that function as molecular chaperones to prevent and repair deleterious damages caused to proteins by environmental and physiological stresses **(Shabtay and Arad, 2005 and Reddy et al.,2008)**.

Molecular chaperones are able to inhibit the aggregation of partially denatured proteins and refold by using the energy of ATP **(Kim et al., 2004 and Giffard et al., 2008)**.

Heat-shock proteins represent cell protective and antioxidant system that may be induced by hyperthermia **(Fehrenbach et al., 2000 and Simpson et al., 2004)**.

HSP70 belongs to a multigene family, and both constitutive (HSc 70) and stress-inducible (HSP70) members exist **(Lindquist and Craig, 1988 and Welch, 1991 & 1992)**.

Mostafa et al. (1999), reported that the most active group (the number of HSPs inducted and disappeared/ 100 KD) in serum protein dynamics in response to WBH is the 68-79KD group i.e. HSP70 family. In agreement with these findings, **Yu et al., (2008)**, showed that HSP70 family is most closely related to the magnitude and duration of thermal stress.

In the present study, the pronounced elevation of serum HSP70 occurred immediately after mild (42°C) and severe (43°C) WBH may be attributed to an increased induction of HSP70 in body cells. The other higher values were induced at 7 days in case of mild WBH (42°C) and at 14, 21 and 28 days in severe (43°C) treatment. In agreement with these findings, **Dinh et al. (2001)** subjected human cells to sublethal thermal injury (55°C for 0-95 min) then returned to the incubator for appropriate recovery period. Proteins were isolated from cells at 1, 4, 8, and 24hr after heating. They found that HSP70 increased at 8 hr and remained elevated beyond 24hr. HSP70 family contains a number of distinct member protein syntheses that include HSP70 or HSP72. This stress is inducible form of HSP70 and HSP73, HSC70. These are the cognate or constitutively expressed from HSP70 and have approximately 80% homology with HSP72 **(Chiang et al., 1989 and Chen and Brown 2007)**.

It is interesting to note that the data in the present study protein polymorphism using SDS-PAGE for serum of hyperthermic male rabbit in both treatments (42°C & 43°C) till the end of the experiment (28 days post-WBH) showed HSP70. This is in agreement with the finding of **Pockley et al. (1998) and Pockley (2001)**, who reported that HSP70 can be detected in the serum of normal individuals. **Yu et al., (2008) and Wang et al.,(2008)** showed that HSP70 family is the most closely related to the magnitude and duration of thermal stress. **Watanabe and Suzuld (1989) and Ralhan and Kaur (1995)** reported that the induction of HSP70 in tumor cells subjected to hyperthermia was less than that in normal or premalignant cells, increased levels of HSP70 correlate with an increase in tumor size. Also, **Englen and Finnel (1991)** using tissue samples showed that the amount of HSP70 produced was increased and directly proportional to the severity of hyperthermic shock and consequently to the amount of thermal damage. Thermal damage to proteins has been proposed as the trigger for activation of heat shock genes resulting in elevated levels of HSP70 **(Edington et al., 1989 ; wang et al., 2007&2008)**. Elevated levels of HSP70 have been related not only to the development of thermotolerance**(Lim et al.,2008)** in mammalian cells, but also to the reduction of thermally induced nuclear damage **(Han et al., 2002)**. **Han et al. (1998)** indicated that cells exposed to HT (60. Hz magnetic fields) continuously for 3 h, HSP70 levels peaked 46% within 20 min and returned to control levels by 2h following a single 20 min exposure. The return of HSP70 levels to control values extended to more than 3h.

Inhibition of HSP70 synthesis enhances the cell death which induced by inhibiting the proteosome which mediates the degradation of ubiquitinated proteins **(Robertson et al., 1999)**.

Nollen et al. (1999) indicated that HSPs in addition to HSP70 are required for removal of heat-induced aggregation of protein from the nucleus. They examined over expression of HSP70 transiently induced using the tetracycline-regulated gene expression system. The in vivo chaperone function of HSP70 could account for recovery of heat-induced elevation in levels of aggregated protein in the cytoplasm but not for recovery of heat-induced increases in levels of aggregated protein in the nucleus (cytoplasmic and nuclear luciferase activity was used to monitor in vivo chaperone function), **(Coss et al., 2002 and Kruse et al., 2008)**.

The induction of HSPs (stress proteins) by stressful stimuli is of itself important in assisting the cell to protect itself from stress. In turn, this leads to the idea that the prior induction of the HSPs by a mild stress or by some other non-stressful procedure would be protective against subsequent more severe stress **(Latchman, 2004)**.

The present results are in agreement with those of **Moustafa et al., (2007)** who reported that induced HSP70 at 0,1,2,4,8,24 and 48hr post WBH using western blotting technique in serum of hyperthermic rabbits, at WBH (42.5°C for 1hr) on female rabbits.

In mammals, testicular temperature is lower than core body temperature. Spermatogenesis to thermal insult has been known for a century, however affected by increases in temperature is not yet clear **(Nakai et al., 2000)**.

Spermatogenesis is a complex process by which spermatogonial stem cell produce sperm, and this process involves remarkable structural and biochemical nature.

Previous studies have identified pachytene spermatocytes and early spermatides as being susceptible to heat stress **(Devita et al., 1990)** and this is not surprising as during meiotic prophase substantial rearrangements of DNA occurs within the nuclei of the leptotene, zygotene and pachytene spermatocytes. They also identified two critical periods in spermatogenesis (leptotene-pachytene and maturation division) through which cells were unable to progress following heating.

The present study showed that the onest of the reaction started early and was more severe in the group treated with 43°C than that kept at 42°C. It is clear that the rabbits which were exposed to 42°C had a mild reaction in the testicular tissue which characterized by mild degeneration, asosperms and giant spermatogonial cells formation. Also showed that the duration time was long throughout the period of the experiment (28 days post heat treatment) with mild changes. Regarding the process of spermatogenesis in this group . While the rabbits which were exposed to 43°C showed severe reaction characterized by degenerative, neurobiotic changes with short duration time until 14 days post heat exposure, and the spermatogenesis was completely affected and nearly stopped then returned back to normal quickly at 21-days and 28 days post heat treatment.

Heat stress (via experimental cryptorchidism and scrotal heating) results in increased apoptosis of testicular germ cell **(Yin et al., 1997; Lue et al., 1999; Yamamoto et al., 2000 and Rockett et al., 2001)**.

The present results are in agreement with those of **Nishiyama et al. (1998)**, who reported that the expression of temperature sensitive RNA binding protein (Cirp) in germ cells was reduced 6 hour after increasing testicular temperature in mice using a water bath (42°C) or by placing the testis in the abdomen.

The results are also in accordance with documented data of **Lue et al. (1999)** who found that short term exposure of the testis to heat causes degeneration of germ cells. They reported that testes of adult male rats exposed to 22°C (control) or 43°C for 15 minutes and killed on days 1,2,9 and 56 after heat exposure and also mild hyperthermia within one or two days resulted in a marked activation of germ cell apoptosis predominantly at early (I-IV) and late (XII-XIV) stages. Stages (V-VI) and (VII-VIII) were, relatively, protected from heat-induced apoptosis.

On day 9, the majority of the tubules were severely damaged and displayed only a few remaining apoptotic germ cells. By day 56, spermatogenesis was completely recovered, and the incidence of germ cell apoptosis was compatible with the control level.

It has been shown that rising temperature can lead to oxidative stress within the rat testis; this is in turn can trigger apoptosis **(Ikeda et al., 1999)**. The effect of oxidative stress on the genomic integrity of spermatozoa has been studied by inducing oxidative stress in the testis and in mature spermatozoa **(Aitken et al., 1989 and Lucesoli and Fraga, 1995)**. Within the testis a number of cell types produce a high level of reactive oxygen species (ROS) that may result in oxidative damage to DNA, proteins and cell membranes and to combat this, the testis has developed a very complex antioxidant system **(Bauche et al., 1994 and Fisher and Aitken, 1997)**.

Therefore, decreased expression of oxidative stress response genes, including extracellular superoxide dismutase $(SOD)_3$, manganese SOD_2 and genes involved in the metabolism of glutathione, which has been reported to occur following heating, may leave cells, more susceptible to oxidative DNA damage **(Barroso et al., 2000; Rockett et al., 2001 and Setchell , 2006))**.

Bankes et al. (2005) observed that the number of apoptotic germ cells remained markedly elevated above controls from 2h to 24 days after the transient heat stress suggesting that the treatment had induced changes in germ cell and/or somatic cell function that had a long term impact on testicular function.

Heat shock proteins (HSPs) are required for spermatogenesis and also protect cells from environmental hazards such as, heat, radiation, and chemicals. Cellular and molecular methods were used to characterize effects of testicular heat shock (43°C for 20 min) at different times post treatment **(Rockett et al., 2001)**. Based on histopathology, spermatocytes was confirmed by TUNEL, and was temporally correlated with the expression of stress inducible HSP70-1 and HSP70-3 proteins in spermatocytes **(Rockett et al., 2001)**.

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