Effect of induced short-term heat stress on functions of liver and kidney of Swiss albinos mice (*Mus musculus*)

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Abstract: Sixty sexually mature, 15 - 22 weeks old, males and femalesSwiss albinos weighing 21 to 43gm each were used to study the effect of heat exposure on their liver and kidney functions. The experimental design was 4 cells of 2 X 2 factorial arrangements for genders and heat treatments. The genders were: 30 male mice, and 30 female mice. The heat treatments were: The Control: 30 mice (15 males and 15 females) kept for four hours at room temperature (25 ± 2 ^{0}C), and The Heat Stress: 30 mice (15 males and 15 females) kept at 35 to 40 ^{0}C ambient temperature for four hours.Levels of the enzymes aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), and total bilirubin (TOTAL BILI) in the serum were used to assess liver functions, while concentrations of serum Urea and Creatinine established the kidney functions.

Mice exposed to 35 to 40^{0}C ambient temperature for four hours were normal during the first one to one and half hour of exposure, after that they became restless, dig in the saw dust, attempted to escape out of the cage, stopped feeding and drinking and their heart beat and respiration rate increased. This continued for another half an hour. After that they became dormant and lied in the corners of the cage until the end of the 4 hours heat exposure. In a separate experiment, mice that were not scarified but returned to normal room temperature after the exposure regained normal activity after 20 to 30 minutes.

All differences in liver and kidney functions between Males and Females control groups were not statistically significant except for ALT which was higher in the Female group than in the Male group.Differences of values of AST, ALP and TOTAL BILI between the control treatments (Males, and Females) were not significant, but those of ALT were significant. All differences of AST, ALT, ALP and TOTAL BILI between the heat exposed treatments (Males and Females) were significant. Significant differences between the control and the heat exposed groups were also encountered. Differences in Urea and Creatinine concentrations between males and females control groups were not significant but were significant between the males and the female's heat exposed groups. Those of the males or females heat exposed groups were significantly different from those of the control groups.

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Introduction:

Hyperthermia or hypothermia in warm-blooded animals is initiated when their core temperature rises or drops above or below the normal limits for which they were designed.Loss of thermia homeostasis may causes short- or long-term multiple organ dysfunction syndromes depending on the level of ambient temperature, duration of exposure and other factors such as relative humidity, air current and physical activity (**Xie*et al*., 2012** and **Cheville, 1999**).Systemic problems include blood, liver and kidney malfunctioning and tissue damage (**Yansen, 2013**; **Liu *et. al*., 2011**; **Saeed *et al*., 2011**; **Xie, 2012**). The extent of these problems is usually measured by blood profile tests such as CBC and liver and kidney function tests, and urine analysis and histological examination of different body tissues. Liver performance is assessed through liver enzyme tests, commonly alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP) and gamma-glutanyltranspeptidaes (GGT), or liver protein tests, usually, total protein, globulin, albumin and bilirubin. Kidney function tests are mainly, serum creatinine, blood urea and electrolytes such as sodium, potassium, chloride……ions.

Studies on hyperthermia commonly use farm animals, pets and mice as experimental subjects. Mice has the advantage of having constant genetic characteristics, small size, fast growth, short life cycle, high fecundity, short gestation period, a breeding season that extends all through the year and easy raising, breading, storing and manipulation in the laboratory. In mice, when the body temperature increased beyond 40 °C, the animal would wet the fur, show signs of dehydration and even irreversible stress which may be followed by death (**Yansen, 2013**).

Most studies on hyperthermia examined effects of long-term exposure of male animals to low heat doses. The objective of the present work is to study the effect of exposing males and females Swiss albino mice (*Mus musculus*) torelatively high heat dose for a short period.

Materials and Methods:

Raising the experimental mice

The present study was conducted at the Zoology Department of Omar Al-Mukhtar University, Albaida, Libya, during September 2015 to April 2016. Sixty sexually mature, 15–22week-old, male and female Swiss albinos weighing 21 to 43 g each were used.

The experimental design

The experimental design (Fig. 1) was a 2 X 2 factorial arrangement for **genders** and **heat treatments**.

The genders were:

- Males and
- Females.

The heat treatments were:

- **The Control:** mice kept for four hours at room temperature, and
- **The Heat Stress:** mice kept at 35 to 40 ^{0}C for four hours.

The variables measured were:

- **Liver function** usingserum Aspartate Transaminase (AST), Alanine Transaminase (ALT), Alkaline Phosphatase (ALP), Total Bilirubin (TOTAL BILI.) and
- **Kidney function** using serum Urea and Creatinine.

The details of the experiment were as follows:

A- Group A. The control males:15 male mice were taken out of the rearing cages weighed individually, marked with colored marks and placed in three cages. The cages were then kept in a small compartment (169 X 116 X 202cm) having ambient temperature of 25 ± 2 ^{0}C (room temperature), then after 4 hours thethroat of each mouse was cut on its side with a sharp razor and the exiting blood was collected in separate labeled heparinized vials that were used for liver and kidney tests.

B- Group B. The control females:15females were taken out of the rearing cages weighed individually, marked with colored marks and placed in three cages. They were then treated as described for Group A.

C- Group C. The heat stressed males:15 male mice were kept for 4 hours in 35 to 40 ^{0}C temperature controlled compartment (169 x 116 x 202cm) to induce the heat stress. The heat was generated by two electric heaters and distributed by an electric fan. Then blood was collected from each mouse as described for Group: A.

D- Group D. The heat stressed females. 15 females were kept for 4 hours in 35 to 40 ^{0}Ctemperature controlled compartment (169 x 116 x 202cm) to induce the heat stress. Then blood was collected from each mouse as described for Group: A.

Gender	Treatments	
	Control **25 ± 2 ^{0}C (room temperature)**	**Heat stressed** **35-40^{0}C**
Males	**Group A**(15 mice)	**Group C**(15 mice)
Females	**Group B** (15 mice)	**Group D**(15 mice)

Fig. 1. The experimental design.

The liver and Kidney function tests:

Blood-containing vials of Group A, B, C and D were taken to "Al-Razi Laboratory for Medical Analysis" where activities of the enzymes (AST), (ALT), (ALP), (TOTAL BILI), Urea and Creatininewere measured using Spectro-Photometer 4040V5+ chemical analyzer.

Results:

The liver Functions

Levels of AST, ALT, ALP and TOTAL BILI activity in the different experimental groups are shown in Table 1 and Figs. 1, 2, 3 and 4. Levels of AST, ALP and TOTAL BILI of male and female controlee groups were not significantly different, but those of ALT were significantly different (Table 1 and Fig.3). All differences of AST, ALT, ALP and TOTAL.BILI between the heat exposed treatments (Male/Female) were significant. AST, ALP and TOTAL.BILI values of Male heat exposed were significantly different from that of Female heat exposed and that of Male or Female control treatments.

Table 1. Values of liver function indicators ± SE (AST, ALT, ALP and TOTAL BILI) of Control and heat exposed Groups.

Sex–Temp. effect	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	TOTAL BILI (mg/dl)
NR	69 -191	26 -120	44 -118	0.3 -0.8
Male control	47.67 ± 3.95a	39.00 ± 3.63a	23.60 ± 3.85a	0.55 ± 0.06a
Female control	48.93 ± 1.64a	52.20 ± 1.21b	27.67 ± 1.03a	0.44 ± 0.04a
Male heated	176.9 ± 8.86b	122.5 ± 7.96c	116.5 ± 6.39c	1.37 ± 0.07b
Female heated	253.5 ± 8.07c	86.07 ± 2.78d	71.13 ± 4.87b	1.71 ± 0.14c

Different superscripts within columns differed significantly ($p \leq 0.05$).

AST aspartate transaminase, ALT alanine transaminase, ALP alkaline phosphatase and total bilirubin. NR: normal values reported in the literature for albino mic

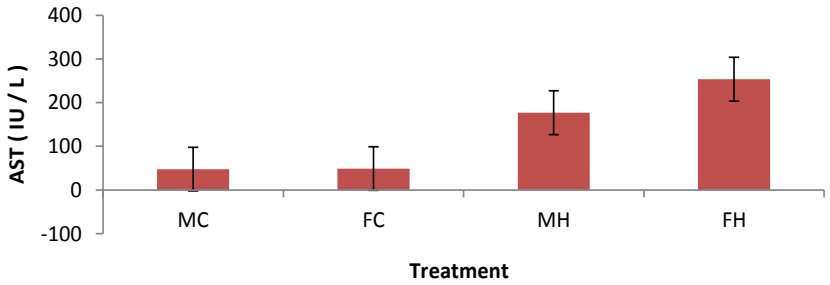

Fig. 2. The effect of heat exposure for 4hrs on AST of male and female mice. The values are mean ± SE.

MC = Male control. FC = Female control. MH = Male heat exposed. FH = Female heat exposed.

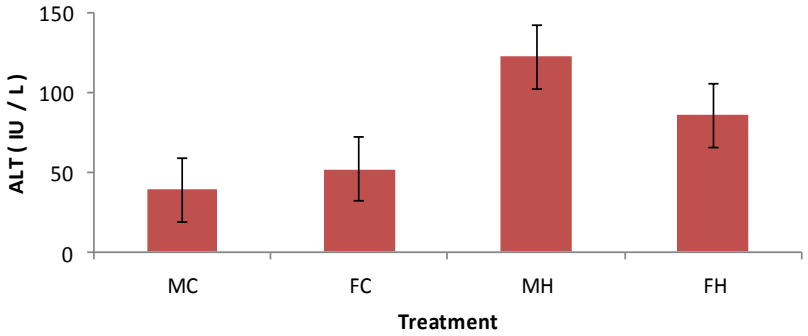

Fig. 3. The effect of heat exposure for 4hrs on ALT of male and female mice. The values are mean ± SE.

MC = Male control. FC = Female control. MH = Male heat exposed. FH = Female heat exposed.

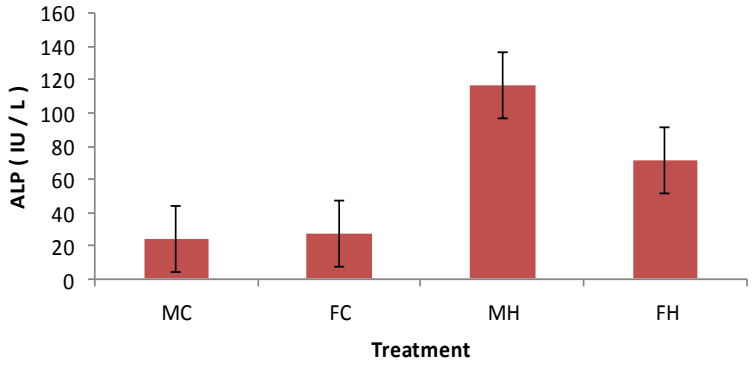

Fig. 4. The effect of heat exposure for 4hrs on ALP of male and female mice. The values are mean ± SE.

MC = Male control. FC = Female control. MH = Male heat exposed. FH = Female heat exposed.

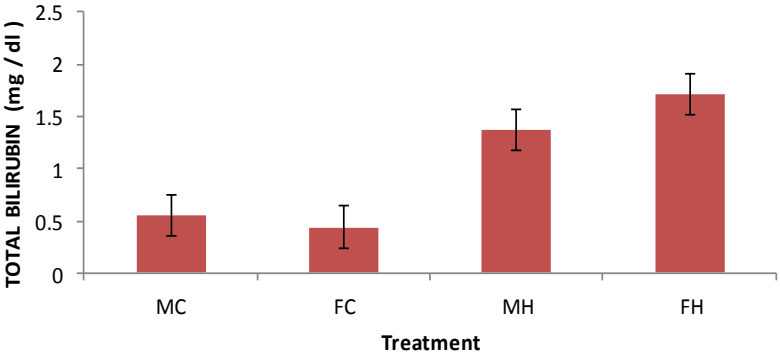

Fig. 5. The effect of heat exposure for 4hrs on TOTAL BILIRUBIN of male and female mice. The values are mean ± SE.

MC = Male control. FC = Female control. MH = Male heat exposed. FH = Female heat exposed.

The kidney Functions

Differences in Urea and Creatinine concentrations between male and female control groups were not significant (Table 2 and Figs. 6 and 7) but were significant between the male and female

heat exposed groups. Those of the male or female heat exposed groups were significantly different from those of the control groups.

Table 2. Values of kidney function indicators ± SE (Urea and Creatinine) of control and heat exposed Groups.

Within columns, means carrying different superscripts differed significantly (p≤0.05).

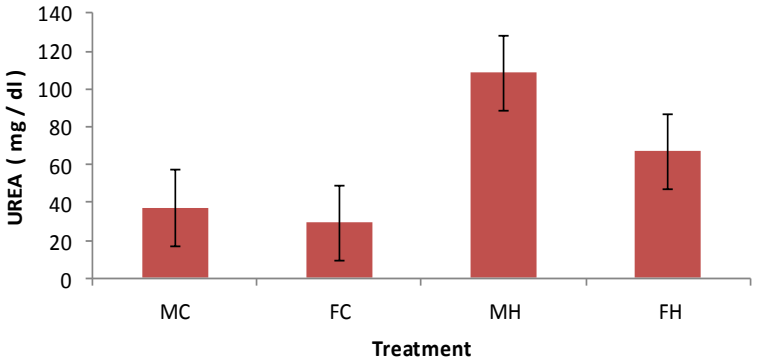

Fig. 6. The effect of heat exposure for 4hrs on UREA of male and female mice. The values are mean ± SE.

MC = Male control. FC = Female control. MH = Male heat exposed. FH = Female heat exposed

Sex-Temp. effect	Urea (mg/dl)	Creatinine (mg/dl)
NR	**19 -34**	**0.5 -0.8**
Male control	**36.93 ± 2.43a**	**0.51 ± 0.05a**
Female control	**29.07 ± 1.27a**	**0.39 ± 0.04a**
Male heated	**108.4 ± 7.71c**	**1.51 ± 0.05c**
Female heated	**66.87 ± 4.11b**	**1.32 ± 0.08b**

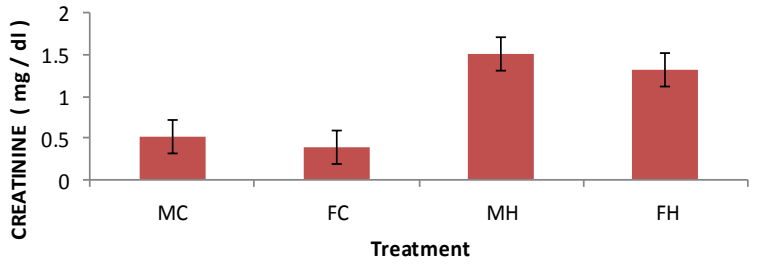

Fig. 7. The effect of heat exposure for 4hrs on CREATININE of male and femalemice. The values are mean ± SE.

MC = Male control. FC = Female control. MH = Male heat exposed. FH = Female heat exposed.

Discussion

Liver functions

Heat exposure produced significant high elevation in enzymes of the liver (ALT, AST, ALP and TOTAL.BILI.) in males and females mice as compared to male and female control groups. These results are in agreement with**Okab*et al.*(2008); Abd El-Rahim *et al*.(2012); and Agrawal and Gupta(2013)**. **Mostafa *et al.* (2002); Mostafa, *et al.*(2007); Abd El-Rahim *et al*. (2012); Easa and Hekal (2015)** pointed out that such an elevated liver enzyme activity may indicate hepatocellular damage which may be a combined result of the high temperature and severely reduced blood Supply **(Rubel, 1984; Abd El-Rahim *et al.*, 2012; Easa and Hekal, 2015)** .The increase of AST and ALT observed after heat exposure could also be related to the net and transient increase in cortisol levels following stress **(Zahran, 2004; Abd El-Rahim *et al.*, 2012; Easa and Hekal, 2015)**. In addition, this elevation could potentially be attributed to the release of these enzymes from the cytoplasm into the blood circulation after rupture of the plasma membrane and cellular damage. Serum AST and ALT are biomarkers in the diagnosis of hepatic damage because they are released into the circulation after cellular damage. These effects may be coupled with a marked hepatic oxidative stress indicating liver injury **(Naik and Panda, 2007; Abd El-Rahim *et al.*., 2012; Easa and Hekal, 2015)**.

Serum ALT, AST, and ALP are the most sensitive markers of liver damage because they are cytoplasmic in location and are released into the circulation after hepatocellular damage **(Sharma, 1997; Pari and Amali, 2005)**. High levels of ALT are indicative of liver injury; AST rises dramatically in acute liver damage **(Reyers, 2003)**. High levels of AST and ALP along with high ALT levels are also indicative of liver injury **(Reyers, 2003)**.

Kidney functions

Serum uric acid, urea and creatinine are often regarded as reliable markers of renal function status **(Henry, *et al.*., 1982; Bonsnes and Taussky, 1982)**. Urea is the detoxification product of the ammonia derived from deamination of amino acids, thus urea is considered to be the end product of protein catabolism **(Sylvia and Mader, 1998)**. Creatinine is a catabolic end product, an anhydride of creatine (or phosphocreatinine) produced by loss of water (or phosphoric acid) from the molecule in an irreversible reaction **(Matthews *et al.*, 1997)**. Thus, elevations in the serum concentrations of these markers are indicative of renal injury, simply because healthy kidneys excrete them first by frst. **Miller (1966)** reported that urea and creatinine represent the two main nitrogenous components that are eventually excreted by the kidney, therefore changes in their levels in the blood stream would reflect the insufficiency of kidney tubules or kidney malfunction. Creatinine, the anhydride of creatine, is formed due to fragility in muscle by irreversible non-enzymatic dehydration of creatine phosphate, which is concerned with the energy mechanism of these tissues and serves primarily as a temporary store of energy. On the other hand, serum creatine concentration is a better indicator of glomerular filtration rate **(Khalil *et al.*, 2002)**. The increase in creatinine may indicate changes in kidney function **(Soliman *et al.*, 2000)**.

In the present study exposure to heat produced a significant increase of serum urea and creatinine levels in males and females mice groups as compared to male and female control groups. The increase was more marked in case of the males than in the females. This is in agreement with **(Easa and Hekal, 2015)**. **Gudev and colleagues (2010)** suggested that increasing plasma urea level in heat stressed buffaloes was closely related with the dynamic of cortisol and blood volume fluctuation in animals under heat. Under heat stress, animals experience abnormal renal function

due to change in the power of selective reabsorption of kidney tubules leading to the retention of urea within the blood **(Abdelatif and Modawi, 1994)**.

Conclusions

- All differences between Males and Females control groups were found to be not statistically significant except for ALT which was higher in the Female group than in the Male group.
- Differences in values of AST, ALP and TOTAL.BILI between the control treatments (Males and Females) were not significant, but those of ALT were significant. All differences in AST, ALT, ALP and TOTAL.BILI between the heat exposed treatments (Males and Female) were significant.
- Differences in Urea and Creatinine concentrations between male and female control were not significant but were significant between the male or female heat groups exposed groups. Those of the male or female heat exposed groups were significantly different from those of the control groups.

Recommendations

- Repeat the present study using longer periods of heat exposure.
- Repeat the present study using parameters that assess effect of exposure on oxidants, immunity, thyroid gland and reproduction.
- Repeat the present study on farm and domestic animals

References:

Abdelatif, A. M. and Modawi. S. M. (1994). Effects of hyperthermia on blood constituents in the domestic rabbits (Lepus cuniculus). J. Therm. Biol. 19 pp: 357-363.

Abd El-Rahim, A. H., Sally, S. A. and Abd El Raouf, A. (2012). Genetic and biochemical studies on hepatocytes of young and old heat-stressed rats, Journal of the Arab Society for Medical Research, 7: pp78–85.

Agrawal, S., and Gupta, D. (2013) .Assessment of Liver Damage in Male Albino Rats after Repetitive Heat Stress of Moderate Level, National Journal of Physiology, Pharmacy & Pharmacology 3 (2) , pp:147 – 152.

Bonsnes, R. and Taussky, H. H. (1982). Determination of creatine and creatinine. In: Fundamental of Clinical Chemistry. Tietz, N.W. (ed.). Saunders Co., Philadelphia, London, Toronto. P: 994.

Cheville, N. F. (1999). Introduction to Veterinary Pathology, 2nd edition. Iowa State University Press, Ames, Iowa.

Easa, F. M., and Hekal, A. M. (2015), Effect of early heat exposure on some physiological and histological changes in the liver and kidney of rabbits before weaning, Egyptian Poultry Science Journal, Egypt. Poult. Sci. (35) (I) pp: 149-175.

Gudev, D., Popova-Ralcheva, S., Moneva, P., Aleksiev, Y., Peeva, T., Ilieva, Y., and Penchev, P. (2010). Effect of heat-stress on some physiological and biochemical parameters in buffaloes. Italian J. Anim. Sci., 6 (2) pp: 1325-1328.

Henry, A. J., Sobel, C. and Kim, J. (1982). Determination of uric acid. In: Fundamental of Clinical Chemistry. Tietz, N.W. (ed.). Saunders Co., Philadelphia, London, Toronto. P: 999.

Khalil, F. S., Marzook, M., Abd-Elmalak, R. H., Hassan, N. A. (2002). Effects of some dietary growth promoters on some blood biochemical and hormonal concentrations, in male and female rabbits during growing period. In: Egypt. J. Rabbit Sci., 12, PP: 1-12.

Liu, Z., Li, B. L., Tong, H. S., Tang, Y. Q., Xu, Q. L., Guo, J. Q., Su, L. (2011). Pathological changes in the lung and brain of mice during heat stress and cooling treatment World J Emerg Med 2011, 2(1), pp: 50-53.

Matthews, H. R., Preed, R. A., and Miesfeld, R. L. (1997). Biochemistry a short course. Wiley-Liss, U.S.A, p: 255.

Miller, E. S. (1966). Textbook of Clinical Pathology. 7th ed. Baltimore: The Williams and Wilkins. Co.

Mostafa, S. I., Abu-Sinna, G. M.,andHassan, A. I. (2002) . Biochemical and histological changes induced by whole body hyperthermia in rabbits. Egypt J BiochemMolBiol, pp: 75–94.

Mostafa, S. I., Bayomy, M. F. F.,andZahran, N. A. R. M. (2007) . The immediate and late effect of sublethal hyperthermia on some biochemical and histological changes in rabbits. J Egypt GerSocZool; 52(A), pp: 29–48.

Naik, S. R.,and Panda, V. S. (2007).Antioxidant and hepatoprotective effects of Ginkgo biloba phytosomes in carbon tetrachloride-induced liver injury in rodents. Liver Int; 27, pp: 393–399.

Okab, A. B., El-Banna, S. G., Koriem, A. A. (2008). influence of environmental temperatures on some Physiological and Biochemical Parameters of New-Zealand Rabbit Males, Department of Environmental Studies, Institute of Graduate Studies and Research, Alexandria University, Egypt , Slovak J. Anim. Sci., 41(1), pp: 12 – 19.

Pari, L.,andAmali , D. R. (2005). Protective role of tetrahydrocurcumin (THC) an active principle of turmeric on chloroquine induced hepatotoxicity in rats. J Pharm PharmaceutSci; 8(1), pp: 115-123.

Reyers, F. (2003). Liver Enzyme Profiling: What Works and What Does Not. Proceedings of 28th World Congress of the WSAVA. Bangkok.

Rubel, L. R. (1984). Hepatic injury associated with heat stroke. Ann Clin Lab Sci; 14, pp: 130–136.

Saeed Al-Zahrani, Saleh Kandeal, Mohamed Mohany and Gamal Badr. (2011). Effects of vitamin E and thymoquinone on physiological and histological characteristics of heat-stressed male mice. African Journal of Pharmacy and Pharmacology Vol. 5(19), pp2174-2183, 22 November, 2011.Available online at http://www.academicjournals.org/AJPP DOI: 10.5897/AJPP11.632 ISSN 1996-0816 ©2011 Academic Journals.

Sharma, R. K. (1997). Morphological and morphometric studies on Liver in rats subjected to repetetive heat stress. Indian J Med Research, 106, pp: 20-26.

Soliman, A. Z. M., EL - Kady, R. I., EL - Shahat, A. A., and Sedki, M. Z. (2000). Effect of some commercial growth promoters on the growth performance and caecum microbiology of growing New Zealand white rabbits. In: Egypt. J. Rabbit Sci., 10, p: 239-252.

Sylvia, S., and Mader, W. (1998). Biology. 6 thed, McGraw-Hill, New York, p :185.

Xie, J. X., Chen, Z. Z., Tang, J., and Zhang, J. (2012). The anti-heat stress effects and acute toxicity tests of an anti- heat stress prescription. The Journal of Animal & Plant Sciences, 22(4), pp: 970-976.

Yansen Li, Yi Huang, YuanguoPiao, Kentaro Nagaoka, Gen Watanabe, Kazuyoshi Taya and ChunMei Li. (2013). Protective effects of nuclear factor erythroid 2-related factor 2 on whole body heat stress-induced oxidative damage in the mouse testis. Reproductive Biology and Endocrinology 2013, pp11-23. http://www.rbej.com/content/11/1/23

Zahran, N. A. R. M. (2004). Hormonal, biochemical and haematological changes in response to acute hyperthermia in rabbits [PhD dissertation]. Faculty of Science, Monofiya University, Egypt.