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Heat Induced Oxidative Stress and Aberrations in Liver Function Leading to Hepatic Injury in Rats

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ABSTRACT

Exposure to heat stress (HS) elicits systemic and cellular response in experimental animals and humans. The current study was undertaken to determine the effect of HS on liver microstructure and function in rats. A heat simulation chamber with ambient temperature (T_a) 45 ± 0.5 °C and relative humidity (RH) 30 ± 5 per cent was used to expose animals to HS. Rats were categorised as moderately heat stressed (MHS, T_c = 40 °C) and severely heat stressed (SHS, T_c = 42 °C) groups. We observed that with rise in core temperature (T_c) alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) levels were increased but glucose level was decreased in both plasma and liver tissue. Significantly elevated levels of reactive oxygen species (ROS) and nitric oxide (NO) were detected in liver of MHS and SHS animals. Additionally, glutathione disulfide and glutathione (GSSG and GSH) ratio was found to be increased with rise in T_c which suggested saturation in antioxidant capacity of tissue. Furthermore, levels of heat shock proteins (HSPs) and caspases were upregulated upon HS. Results of histological examination indicated extensive loss of cells in liver parenchyma leading to disorganisation of lobular structure. Thus, biochemical and histological studies in experimental animals demonstrates that HS may severely altered structural and biochemical functions of liver.

Keywords: Liver; Heat stress; Liver function test; Oxidative stress; Heat shock proteins

NOMENCLATURE

ALT	Alanine Amino Transferase
AST	Aspartate Amino Transferase
ALP	Alkaline Phosphatase
BCIP	5-Bromo-4-Chloro-3-Indolyl Phosphate
FU	Fluorescence Units
GSH	Glutathione
GSSG	Glutathione Disulfide
PVDF	Poly Vinylidene Di Fluoride
HR	Heart Rate
HS	Heat Stress
HSP	Heat Shock Protein
LFT	Liver Function Test
MHS	Moderate HS
MOD	Multi Organ Dysfunction
NBT	Nitro Blue Tetrazolium
NO	Nitric Oxide
RH	Relative Humidity
ROS	Reactive Oxygen Species
SEM	Standard Error Mean
SHS	Severe HS
T_a	Ambient Temperature
T_c	Core Temperature
T_s	Skin Temperature

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1. INTRODUCTION

Prolonged exposure to high ambient temperature (T_a) causes heat associated illnesses which progressively results in heat cramp, exhaustion and heat stroke. Heat cramp comprises of painful cramps and moist skin whereas heat exhaustion is commonly associated with water and sodium depletion. Heat stroke is a condition where heat dissipation is significantly reduced and is divided into classical and exertional heat stroke. Both stroke conditions develop as core temperature (T_c) reaches 40 °C and above[1-2]. Exertional heat stroke develops rapidly whereas classical heat stroke develops slowly over several days with elevated T_c and is also associated with failure of the central nervous system (CNS) and multi-organ dysfunction (MOD)[3-6]. Major factors that contribute towards the production of HS are humidity, air movement, metabolic rate, clothing and temperature[7]. Cardiac functions are also affected on exposure to environmental stressors which are measured in the form of heart rate (HR) and mean arterial pressure (MAP) of blood[8-10]. In general, HS affects whole body but has severe effects on brain, heart, lungs, intestine, kidney and liver[11].

Liver is the second largest organ of the body which performs more than 500 vital function that are broadly categorised as storage, protein synthesis, metabolism, excretion and detoxification of potent toxins[12]. Liver parenchyma functions as storage for glycogen, fat and fat-

soluble vitamins which are essential for several physiological functions. The synthesis of plasma proteins, clotting factors, urea and glucose occur in the liver which is later released into the blood stream. Bile is synthesised in the liver and helps in removal of toxins and other excretory metabolic by-products from the body[13]. Heat stroke causes widespread tissue injury, if immediate appropriate action is not taken then it results in high mortality[14]. Heat stroke also induces liver damage which was initially reported more than 80 year ago but its mechanism is still unclear[11]. However, alterations in physiological variables and changes in biochemical parameters have been reported by several research groups[15-17]. A set of assays known as liver function test (LFT) are performed to monitor vital functions of the liver[18]. LFT included estimation of ALT, AST, ALP, blood glucose, lactate dehydrogenase (LDH), urea, bilirubin and albumin[19]. Perturbations in the above parameters indicate that liver is not performing normal physiological functions appropriately[18].

Recent reports suggest that heat exposure can induce oxidative damage in mammalian tissue[20-21]. Oxidative stress is defined as an imbalance between production and destruction of ROS via the antioxidant defense system[22]. ROS consisting of superoxide anion (O_2^-), hydroxyl radical and hydrogen peroxide have the potential to trigger oxidative damage of proteins, lipids and nucleic acids. The antioxidant system is affected on exposure to HS, as glutathione (oxidised and reduced state) level shows variation in response to HS[16,23,24]. In addition, HSPs are induced in several tissues in animals exposed to numerous stressors. Numerous research groups have been reported that HSPs have strong cytoprotective effect, act as molecular chaperones and are involved in many regulatory pathways. HSP27, 70 and 90 are predominantly anti-apoptotic[25], whereas HSP60 is pro-apoptotic in nature[26]. It has been shown that HSPs inhibit key steps in an apoptotic cascade and maintain physiological homeostasis which is necessary for cell survival during exposure to environmental stress conditions[15,22,27]. The apoptotic signals (e.g. Fas ligand and tumor necrosis) stimulate caspases and transform pro-caspases (e.g. procaspase-3, 8 and 9) in to active caspases. The cleaved apoptotic substrates alters significant cellular processes and morphology leading to cell death[28-29].

Recent reports demonstrate that rats with increased T_c experience hepatic injury[30-31]. In the present report we study the effect of moderate and severe HS on liver pathophysiology. To accomplish this objective, rats were exposed to HS followed by measurement of physiological and biochemical parameters. In addition, expression level of HSPs and caspases were also analysed along with histological examination of liver parenchymal sections.

2. MATERIALS AND METHODS

2.1 Experimental Animals and Housing Condition for Rats

Sprague dawley rats with average body weight 250 gm - 300 gm were used from the animal house, DIPAS. Rats were maintained in a temperature-controlled room (T_a = 23 ± 2 °C and RH 55 ± 5 %) on a 12/12 h light/dark cycle with food and water available *ad libitum*.

2.2 Experimental Design and Stress Exposure of Rats in Heat Simulation Chamber

Experimental design and heat exposure of conscious rats were in accordance to Institutional Animal Ethical Committee (IAEC/DIPAS/2015-17). Tissue samples from Chauhan[32], *et al*. were utilised to study the effect of hyperthermia on liver structure and functions. In brief, a total of 18 animal were randomly assigned to the following three group with six rat in each group (N = 6). The first group was exposed to T_a = 25 ± 2 °C and RH = 30 ± 5 per cent in heat simulation chamber for 50 - 60 min which served as the control group. The second group was exposed to T_a = 45 ± 0.1 °C with RH = 30 ± 5 per cent till T_c reached 40 °C[33-34] this served as MHS group. Similarly, animals in third group were exposed to T_a = 45 ± 0.1 °C and 30 ± 5 per cent RH till T_c reached 42 °C and were called SHS group.

2.3 Measurement of Physiological Parameters

The major physiological parameter viz. non-invasive blood pressure, HR, T_c and skin temperature (T_s) were measured as per[32,35] all parameters were continuously monitored on a digital display using Lab chart 7 software (AD Instruments).

2.4 Surgery and Sample Collection

All surgical procedure of heat exposed and unexposed control rats were performed under anesthesia with an intra-peritoneal dose of 80 mg/kg ketamine and 5 mg/kg xylazine. Rats were sacrificed, blood (further processed for plasma isolation) and tissue samples were collected followed by snap freezing in liquid nitrogen and stored at - 80 °C for further biochemical and molecular studies.

2.5 Histological Analysis of Rat Liver Tissue

The liver sample of all three group was used to examine morphological changes with perfusion in saline (0.9 % NaCl). For histological examination, dissected tissues were immersed in 10 per cent formalin solution for 10 h followed by immersion in 70 per cent, 90 per cent, and 100 per cent ethanol for 30 min each time. The tissue was finally immersed in xylene for 20 min and embedded in paraffin at 58 °C for microtome sectioning (5 – 15 μm). Sections were floated in water bath at 56 °C and mounted onto gelatin coated histological slides for hematoxylin and eosin (HE) staining. HE staining was performed as per standard protocol[36].

2.6 SDS-PAGE and Immuno-Blotting

For tissue lysate preparation, 100 mg of rat liver was homogenised in 1 ml of PBS containing proteases and phosphatases inhibitor cocktail. Protein estimation of tissue lysate was performed by Bradford method and further used for biochemical and molecular analysis. Equal amount of protein from each sample was loaded and resolved on a vertical 10 cm x 8 cm SDS-polyacrylamide gel at a constant potential gradient of 100 V for 1 - 2 h. Proteins were transferred to Polyvinylidene difluoride (PVDF, 0.45 micron) membrane by semi-dry method at constant volt (11V). Immuno detection was performed by blocking the blots with 5 per cent BSA for 1 h at RT followed by incubation with primary antibody for overnight at 4 °C. The

blots were then washed three time with TBST and once with TBS before incubating with AP conjugated secondary antibody for 2 h at RT and again washed as above. Finally, the blots were developed with AP substrate {(5-Bromo-4-Chloro-3-Indolyl Phosphate (BCIP) and Nitro Blue Tetrazolium (NBT)}.

2.7 Liver Function Test

A total of nine parameters were assessed in rat liver tissue lysate and plasma as per manufacturer's instructions. In brief, ALT and AST were estimated by mixing reagent with samples in a ratio of 10:1 then absorbance was measured at various interval of 1 min, 2 min, and 3 min (Randox, Cat No. AL1205 and AS1204) at 340 nm. ALP was estimated by mixing reagent with samples in a ratio of 50:1 followed by measurement of absorbance at similar interval (Randox, Cat No. AP311) and wavelength. For the estimation of LDH and Urea samples were mixed with reagents in a ratio of 1:25 and 100 respectively, then absorbance was measured at different intervals of 0.5 min, 1 min, 2 min, and 3 min (Randox, Cat No. LD401 and UR221) at 340 nm. Glucose and albumin were estimated by mixing reagents with desired samples in ratio of 100 and 300:1 respectively; the mixture was incubated for 25 min then absorbance was measured (Randox, Cat No. GL2614 and AB362) at 500 mm and 630 nm, respectively. For measurement of direct and total bilirubin samples were mixed with reagents in a ratio of 1:25 followed by incubation for 5 min (Randox, Cat No. BR2361 and BR2362) then absorbance was measured at 546 nm.

2.8 Measurement of Oxidative Stress Markers

2.8.1 Reactive Oxygen Species

For the estimation of ROS, 25 µl of sample was diluted in 1.494 ml of PBS followed by addition of 6 µl Dichloro-dihydro-fluorescein diacetate (DCFHDA, 1.25 mM). The reaction mix was then incubated for 15 min at 37 °C in dark. The fluorescence was measured at 485 nm Excitation and 530 nm Emission[37-38].

2.8.2 Lipid Peroxidation

To measure lipid peroxidation i.e of MDA levels, 150 µl of 20 per cent Trichloroacetic acid (TCA) and 150 µl of 0.67 per cent 2-Thiobarbituric acid (TBA) was added to 50 µl of samples and incubated at 85 °C for 45 min. The samples were then centrifuged at 2000 rpm for 5 min and 200 µl of supernatant was used for measuring absorbance at 531 nm[23].

2.8.3 Protein Oxidation

0.5 ml 10 mM 2,4-Dinitrophenylhydrazine (DNPH) in 2 M hydrochloric acid (HCl) was added to 0.5 ml of sample and the mixture was incubated for 1 h at RT, with vortexing every 15 min. 0.5 ml 20 per cent of TCA was then added followed by centrifugation at 11,000 g. The pellet obtained was washed thrice with 1 ml ethanol-ethyl acetate (1:1) to remove free reagent and was solubilised in 400 µl of 6 M guanidine. The sample blank was incubated with 2 M HCl without DNPH. Protein oxidation estimated by spectrophotometer measurement of hydrozones generated by reaction of DNP (Dinitrophenyl) with protein carbonyl group at 366 nm[39-40].

2.8.4 Measurement of GSSH::GSH Ratio

All reagents, samples and standards were prepared as per manufactures instructions using kit (Abnova, Cat No.: KA3779). 50 µl samples were used for estimation of GSH and GSSG and fluorescence was measured at Ex/Em = 490/520 nm in a fluorescence plate reader.

2.8.5 Nitric Oxide Estimation

To measure production of NO, 100 µl of griess reagent was added to 100 µl sample as well as in standards ($NaNO_2$; 1-20 mM) and was incubated for 15 min at RT. Absorbance was measured at 540 nm. The result was calculated as µmole/g protein and expressed as percentage change with respect to control group.

2.9 Statistical Analysis

Data were statistically evaluated by one-way analysis of variance (ANOVA), using GraphPad Prism (Version 5.0) software. Bonferroni's multiple comparison tests were performed when ANOVA was significant. The values were presented as the mean ± SEM of six animals and the level of significance was set at $P \leq 0.05$.

3. RESULTS AND DISCUSSION

Since exposure to SHS leads to MOD which includes liver injury, we investigated the effect of HS on liver structure and functions in experimental animals.

3.1 Measurement of Physiological Parameters

Physiological parameters measured include T_c, T_s, HR and MAP. As described earlier, rats were exposed to MHS in which T_c reached 40 °C in 15 - 20 min and to SHS in which T_c reached 42 °C within 30 - 40 min. In both forms of stress, HR along with MAP rises with increase in T_c but MAP drops in SHS as compared to rats exposed to MHS[32]. Elevated MAP as observed during exposure to MHS is associated with promotion of vascular injury which ultimately leads to multi organ failure. In contrast, low blood flow was observed in vital organs due to decreased MAP on exposure to SHS condition[33,41]. As discussed in previous reports HS alters blood flow distribution toward cutaneous circulation with decreased flow in splanchnic and renal area resulting from vasoconstriction[42]. In view of the above, our observations suggest that animals exposed to both MHS and SHS are under stress and at risk of MOD[43-44]. We next examined liver functions in rats exposed to MHS and SHS state.

3.2 Liver Enzyme Levels were Increased But Glucose Level was Decreased Under HS

Extracted plasma and tissue lysate were used to determine LFT parameters in MHS and SHS rats as compared to control. Plasma samples (2 - 10 µl) were used for determination of each LFT parameter. Plasma levels of ALT ($P_p \leq 0.01^{MHS}$, 0.01^{SHS}, Fig. 1(A)), AST ($P_p \leq 0.01^{SHS}$, Fig. 1(C)), ALP ($P_p \leq 0.05^{MHS}$, 0.01^{SHS}, Fig. 1(E)), LDH ($P_p \leq 0.05^{MHS}$, 0.05^{SHS}, Fig. 1(K)), Urea ($P_p \leq 0.01^{SHS}$, Fig. 1(G)), total bilirubin ($P_p \leq 0.05^{SHS}$, Fig. 1(O)) and direct bilirubin ($P_p \leq 0.05^{SHS}$, Fig. 1(Q)) were all significantly elevated in rats exposed to either MHS or SHS.

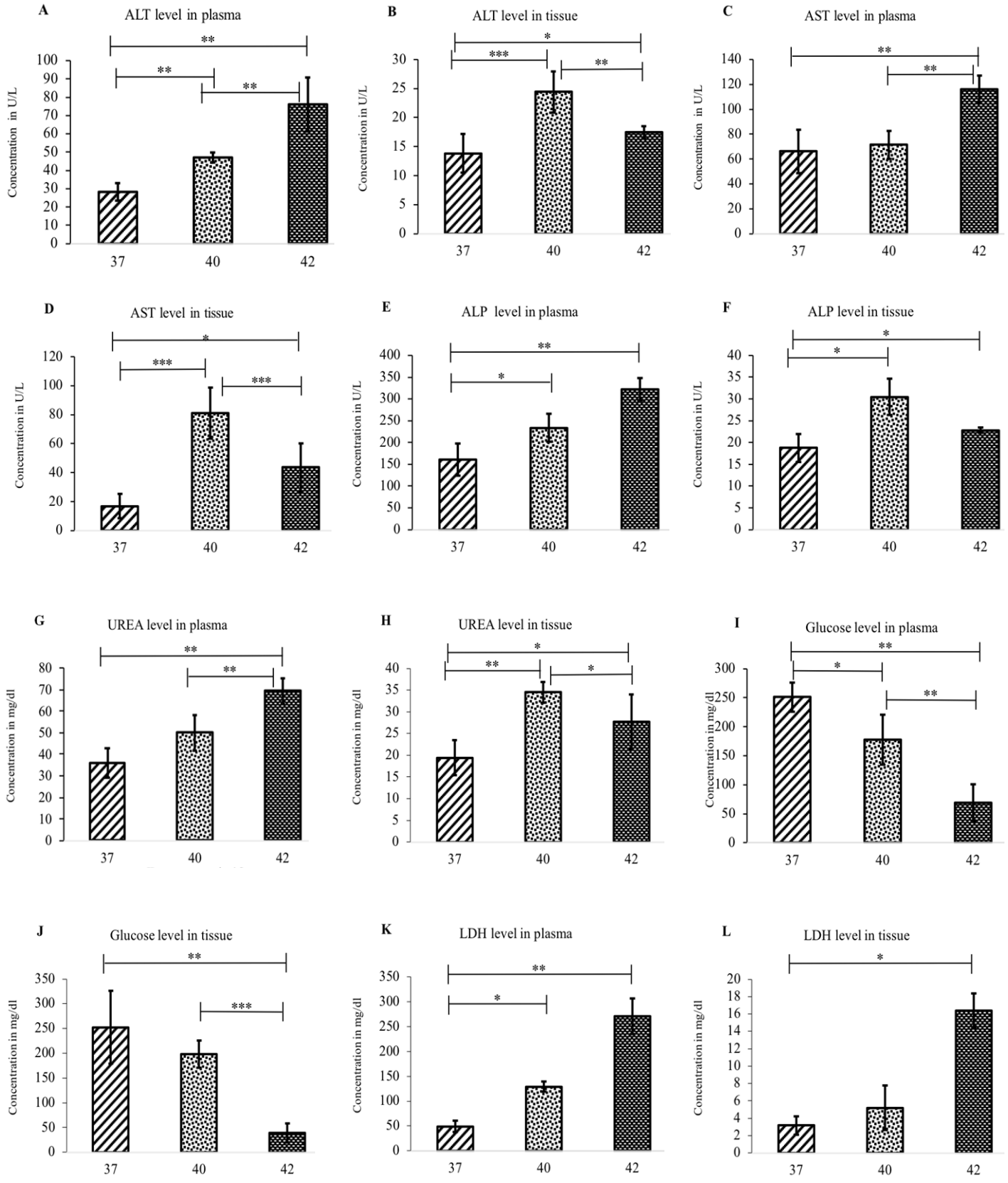

Figure 1. Measurement of LFT parameters in rat plasma and liver tissue lysate on exposure to MHS and SHS compared with unexposed control. ALT in plasma (A) and in tissue (B); AST in plasma (C) and in tissue (D); ALP in plasma (E) and in tissue (F); Urea in plasma (G) and in tissue (H); Glucose in plasma (I) and in tissue (J); LDH in plasma (K) and in tissue (L) Values are represented in mean ± SEM (N=6). * P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001 for plasma and tissue samples; unit of concentration of respective LFT parameter is shown (in U/L or mg/dl). All parameters were examined using Randox kits and protocols were followed as per manufacturer's manual.

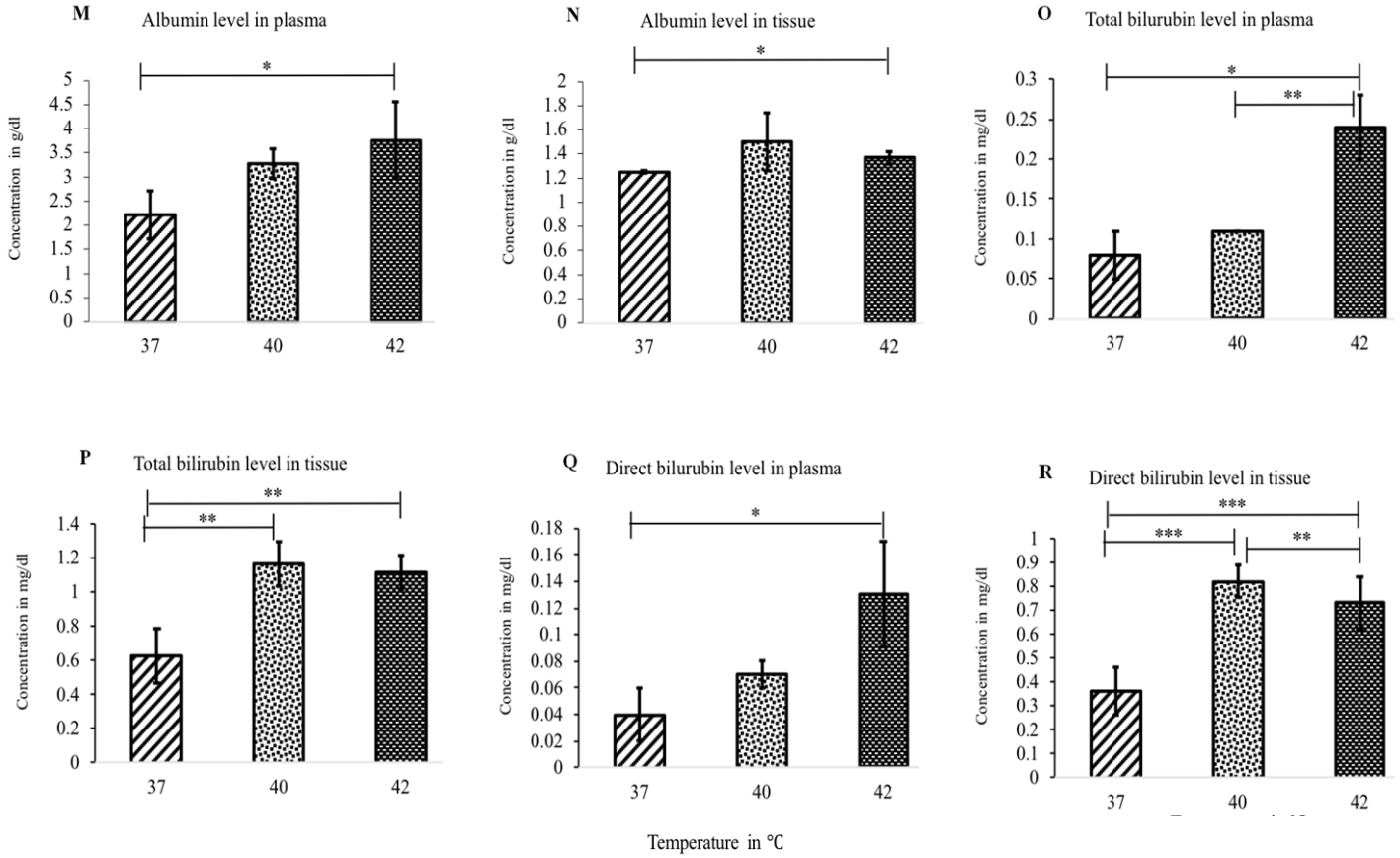

Figure 1. Measurement of LFT parameters in rat plasma and liver tissue lysate on exposure to MHS and SHS compared with unexposed control. Albumin in plasma (M) and in tissue (N); Total bilirubin in plasma (O) and in tissue (P); Direct bilirubin in plasma (Q) and in tissue (R). Values are represented in mean ± SEM (N=6). * P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001 for plasma and tissue samples; unit of concentration of respective LFT parameter is shown (in U/L or mg/dl). All parameters were examined using Randox kits and protocols were followed as per manufacturer's manual.

However, glucose levels were found to be decreased ($P_p \leq 0.05^{MHS}$, 0.01^{SHS}, Fig. 1I) under similar conditions in rat plasma samples (Fig. 1). To confirm that the alterations in parameters as indicated above were due to alterations in liver function and not due to interference from other organ(s), we further measured all the above parameters in liver tissue homogenate (working protein concentration 25 mg/ml). Similar results were observed in tissue lysate as compared with plasma samples. In the tissue homogenate levels of ALT ($P_t \leq 0.001^{MHS}$, 0.05^{SHS}, Fig. 1(B)), AST ($P_t \leq 0.001^{MHS}$, 0.05^{SHS}, Fig. 1(D)), ALP ($P_t \leq 0.05^{MHS}$, 0.05^{SHS}, Fig. 1(F)), LDH ($P_t \leq 0.05^{SHS}$, Fig. 1(L)), Urea ($P_t \leq 0.01^{MHS}$, 0.05^{SHS}, Fig. 1(H)), total bilirubin ($P_t \leq 0.01^{MHS}$, 0.01^{SHS}, Fig. 1(P)) and direct bilirubin ($P_t \leq 0.001^{MHS}$, 0.001^{SHS}, Fig. 1(R)) were significantly elevated in rats exposed to either MHS or SHS and glucose levels ($P_t \leq 0.01^{SHS}$, Fig. 1(J)) were also decreased. Albumin was also found to be increased with similar pattern in tissue ($P_t \leq 0.05^{SHS}$, Fig. 1(N)) and plasma ($P_p \leq 0.05^{SHS}$, Fig. 1(M)).

ALT, AST, ALP, and LDH are synthesised by several organs including the liver. Thus, increased levels of these enzymes in plasma along with tissue suggest liver damage with cholestatic pattern of biliary pathology in rats exposed to MHS and SHS. Since ALP is a membrane bound enzyme, it functions as marker for cellular membrane damage in animals exposed to HS. Elevated level of ALP also indicated inhibition of bile excretion of liver[45]. More than 1.5 time increased ratio of AST:ALT (De Ritis ratio) suggests presence of extra hepatic obstruction in liver. Bilirubin, a by-product of haemoglobin breakdown was also found to be increased in HS that suggests increased haemolysis as well as obstruction of hepatocytes and biliary tree function[19,46]. All these symptoms indicate that there is severe cholestasis in liver due to HS[18]. Albumin is primarily synthesised in liver and found to be increased in hyper-albuminemia state which are involved in dehydration and hyper-osmotic pressure[47]. In this condition transportation of various bio-molecules to destined tissues are drastically affected. This indicates that during HS the osmoregulatory system of organ is disrupted[19]. Recent reports have suggested that HS modulates glucose metabolism by lowering energy status of the cell. We also observed decreased level of glucose (Figs. 1 (I) and (J)) in plasma and liver tissue exposed to HS also suggest alteration in carbohydrate metabolism[48]. Previous investigations have recommended that lactate metabolism is upregulated but at the same time glucose levels were also decreased[49]. Our data also suggest the same that LDH is upregulated and glucose levels were decreased on exposure to MHS and SHS rats. Specifically, increased levels of above LFT parameter of plasma are comparable with increasing T_c.

The possible explanation for this could be that these markers are also secreted from other organs apart from liver.

3.3 Redox Imbalance in Rat Liver Tissue Exposed to HS

A complex antioxidant system is present in mammals to protect cells from ROS that are produced both under normal as well as during exposure to stress conditions[50]. ROS is generated as a by-product of oxygen metabolism which is upregulated in animals on exposure to HS[21,50]. Previous investigators have also shown that NO level alters in animals exposed to stress conditions[51]. We observed high level of ROS ($P_t \leq 0.001^{MHS}$, 0.001^{SHS}, Fig. 2(A)) and NO ($P_t \leq 0.05^{MHS}$, 0.001^{SHS}, Fig. 2(D)) in MHS and SHS tissue as compared to control. In addition to the above data we also found increased lipid peroxidation (MDA production $P_t \leq 0.01^{MHS}$, 0.001^{SHS}, Fig. 2(E) and protein oxidation ($P_t \leq 0.01^{MHS}$, 0.01^{SHS}, Fig. 2(B)) with rise in T_c. Recently, investigators have reported that protein synthesis is affected by increased lipid peroxidation and protein oxidation was also shown to be higher in animals exposed to HS[50,52-53]. In previous reports it has been shown that during HS, level of glutathione decreases and glutathione disulphide increases in animal tissue[24,50]. In our investigation we measured level of both GSH and GSSG and it was observed that GSH goes down while GSSG goes up upon exposure to HS. Next, we calculated the ratio of GSSG:GSH which is upregulated ($P_t \leq 0.05^{SHS}$, Fig. 2(C)) with rise in T_c, suggesting that there is a decrease in potential to combat oxidative stress during HS. Excess production of ROS, RNS, lipid peroxidation and protein oxidation upon exposure to HS promotes cellular degeneration and cell death leading to liver injury in rats[54] as shown in Fig. 2.

3.4 Heat Induced Increased Expression of HSPs and Caspases in Tissue Lysate

HSPs are major players in the regulation of proteostasis and signal transduction pathways during exposure to numerous stress conditions. On exposure to HS expression of HSPs and HSF1 were found to be altered in liver. The protein expression data showed significantly increased levels of HSP70 ($P_t \leq 0.05^{MHS}$, 0.05^{SHS}, Figs. 3(A) and 3(B)), HSP90 ($P_t \leq 0.05^{MHS}$, 0.05^{SHS}, Figs. 3(A) and 3(C)), HSP105 ($P_t \leq 0.01^{MHS}$, 0.01^{SHS}, Figs. 3(A) and 3(D)) and heat shock factor HSF1 ($P_t \leq 0.01^{MHS}$, Figs. 3(A) and 3(E)) in MHS and SHS rats. Apoptotic marker caspase 3 ($P_t \leq 0.05^{MHS}$, 0.05^{SHS}, Figs. 4(A) and 4(B)) and Caspase 9 ($P_t \leq 0.05^{MHS}$, 0.05^{SHS}, Figs. 4(A) and 4(C)) also showed upregulation in MHS and SHS liver tissue. HSPs are known to play a cytoprotective role in cells and tissues exposed to various chemical and environmental stressors[55]. Reports suggest that HSF1 is essentially required for activation of these HSPs[56-57] which further interact with many cellular proteins including cysteine proteases. Caspases are cysteine proteases which interact with HSPs and participates in various stress conditions[21,27,28]. During exposure to HS interplay between

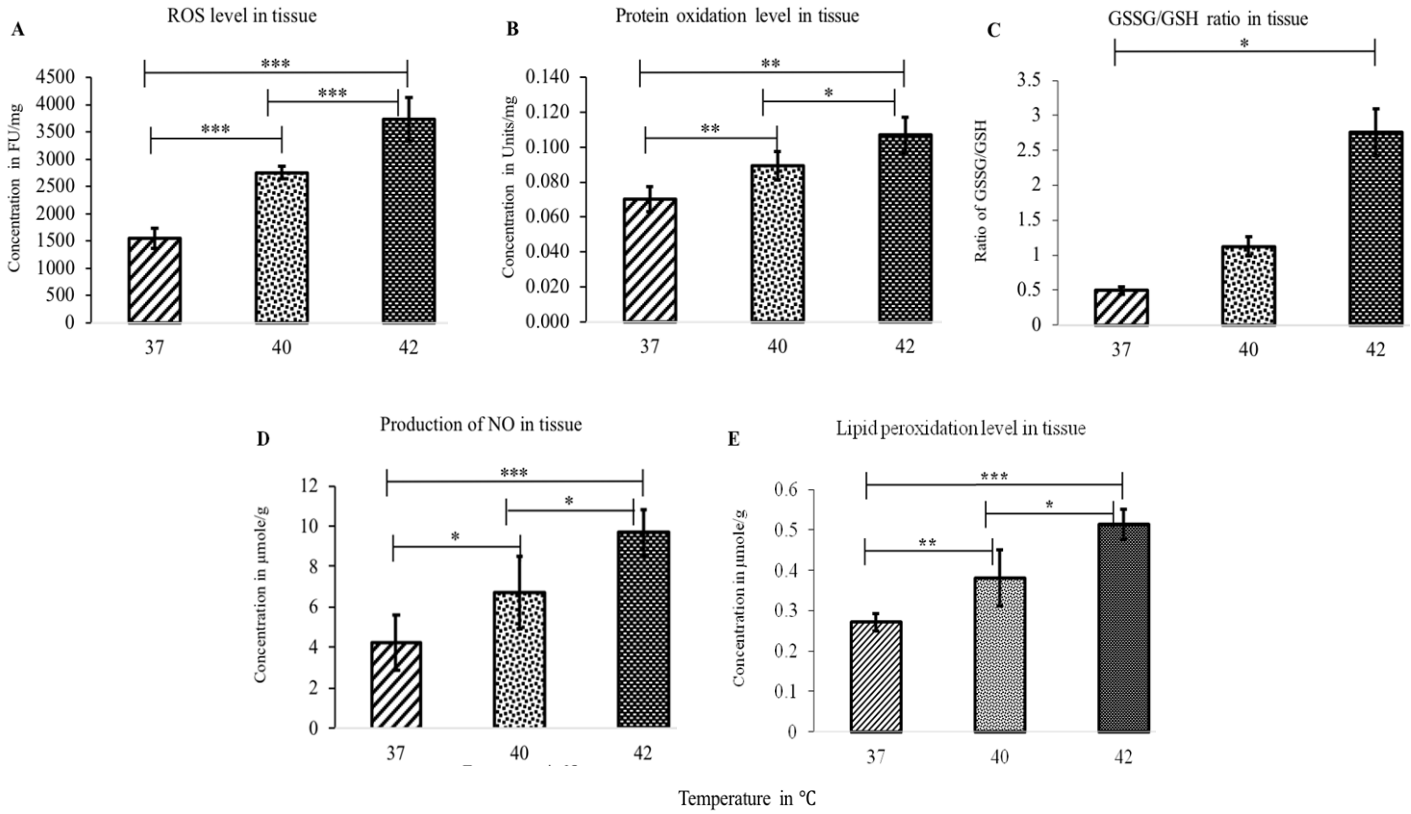

Figure 2. Analysis of oxidative stress markers in liver tissue lysate exposed to HS (A) ROS level (B) Protein oxidation level (C) Ratio of GSSG/GSH (D) NO level (E) Lipid peroxidation (MDA production) level. Values are represented in mean ± SEM (N=6). * $P_t \leq 0.05$, ** $P_t \leq 0.01$, * $P_t \leq 0.001$; where unit of concentration of respective oxidative stress parameter shown in graph was normalised with respect to protein concentration for each sample.**

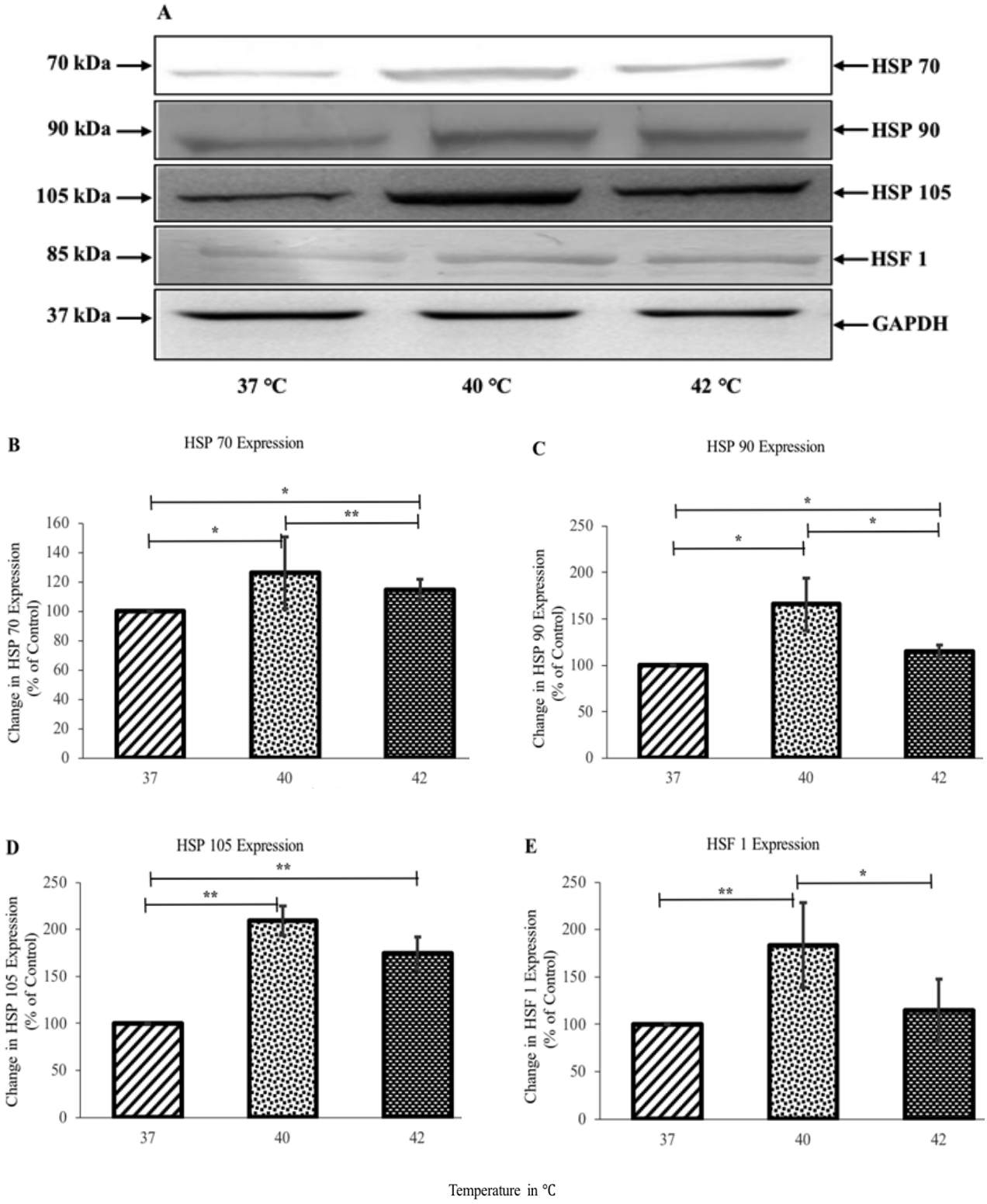

Figure 3: Effect of heat exposure on HSP expression in liver tissue lysate by western blotting and immuno-detection. In brief, frozen liver tissue was homogenised in RIPA buffer containing proteases and phosphatases inhibitors; a total 60 µg protein was resolved on 8-10% SDS-PAGE and run as per standard protocol for 1-2 h at constant volt. Proteins were transferred on PVDF membrane; blots were first probed with antibody of respective proteins and developed with alkaline phosphatase-conjugated anti-rabbit IgG and alkaline phosphatase substrates (BCIP and NBT). Representative data are shown as (A) Protein expression of HSP70, HSP90, HSP105, HSF1 and GAPDH as protein bands. Ratio of intensity of (B) HSP70 expression (C) HSP90 expression (D) HSP105 expression and (E) HSF1 expression. Analysis of densitometry scans was carried out using ImageJ software with results from triplicate independent experiments for N = 6 in each condition, average data are shown in SD. Results were normalized to 100 for unexposed control. GAPDH was used as loading control. Bars are the mean ± SEM of six animals per group. * $P_t \leq 0.05$ $P_t \leq 0.01$, *** $P_t \leq 0.001$.**

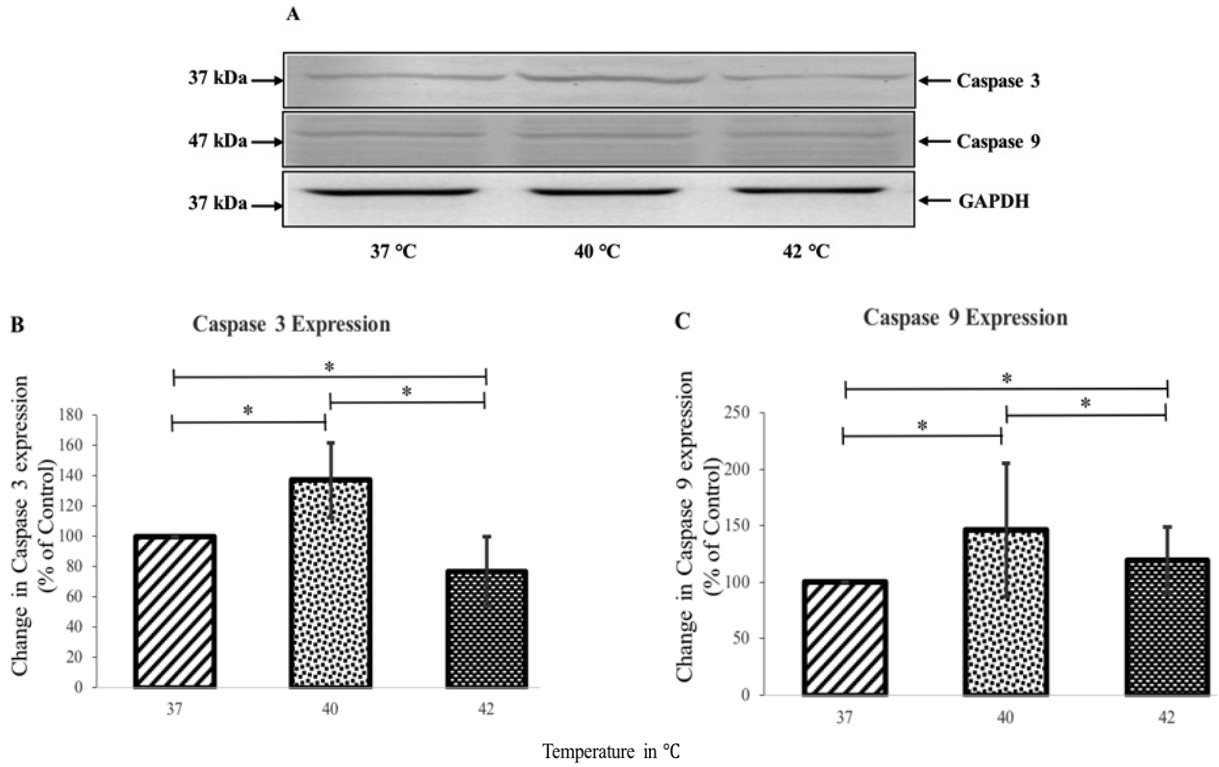

Figure 4. Effect of thermal stress on expression of caspases in liver tissue lysate by western blotting and immuno-detection. Sample preparation was performed as described in Figure 3. Representative data are shown as (A) Protein expression of caspase 3, caspase 9 and GAPDH as protein bands. Ratio of intensity of (B) Caspase 3 expression (C) Caspase 9 expression. Analysis of densitometry scans were carried out using ImageJ software with results from three independent experiments for N = 6, average data are shown in SD. Results were normalized to 100 for unexposed control. GAPDH was used as loading control. Bars are the mean ± SEM of six animals per group. * $P_t \leq 0.05$ $P_t \leq 0.01$, *** $P_t \leq 0.001$.**

increased levels of caspase 3 and caspase 9 (Fig. 4) that induce cell death by apoptosis and elevated levels of HSPs that provide cytoprotective function which determine cell fate. It has been observed that expression of caspases and HSPs were increased in MHS with respect to control due to heat stress but it was downregulated in SHS with respect to MHS. It was expected that expression of these proteins might have gone higher in SHS state with respect to MHS. In contrast, our results showed decreased expression in SHS with respect to MHS; however it was higher as compared to unexposed control. The possible

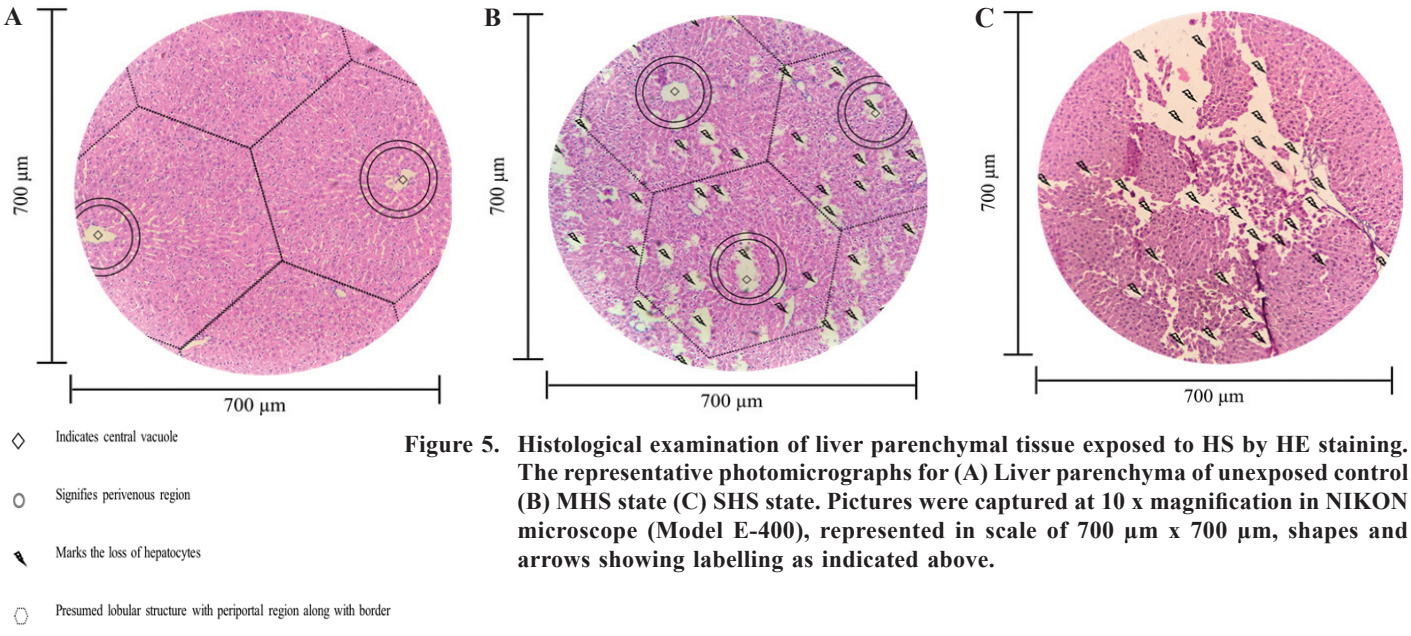

Figure 5. Histological examination of liver parenchymal tissue exposed to HS by HE staining. The representative photomicrographs for (A) Liver parenchyma of unexposed control (B) MHS state (C) SHS state. Pictures were captured at 10 x magnification in NIKON microscope (Model E-400), represented in scale of 700 µm x 700 µm, shapes and arrows showing labelling as indicated above.

explanation for this could be heat induced altered protein synthesis[58], redox imbalance and cell death along with higher T_c[52]. Protein expression data shows that HSP functions as cytoprotective agents in MHS rats but in SHS same function declines and unable to sustain the integrity of proteins. The caspases expression data indicated that there is an activation of apoptotic pathway.

3.5 Histological Examination of Liver Parenchyma in Heat Exposed Rats

So far, the study has presented alteration in markers of liver functions, redox imbalance and up-regulation of cytoprotective proteins and caspases in heat stressed rats. Thus, further investigation is required to study the basic architecture of liver exposed to HS with respect to unexposed control. Liver sections were stained with HE as per standard protocol and observed under light microscope. The dotted line denotes lobular region, diamond represents central vacuole and concentric ring marks the privenous region as shown in Fig. 5. Recent studies indicated structural alteration and enhanced injury in liver due to various pathophysiological states such as alcohol induced-fatty liver disease[31], heat-induced liver damage[14,30]. We also observed liver damage in both MHS and SHS animals as compared to unexposed control as shown in Fig 5. During MHS rat liver parenchyma shows moderate loss of liver cells, indicated (spots where loss of parenchyma) by arrowhead due to mild effect of HS as shown in Fig. 5(B). In SHS rat liver, the lobular structure cannot be identified due to heavy distortion in orientation of hepatic lobule as shown in Fig. 5(C). Thus, morphological investigation indicated that SHS state parenchymal damage was higher than MHS animals which were unable to be recovered by regeneration power of liver.

4. CONCLUSIONS

Findings from biochemical and microscopic studies conclude that there is severe functional and structural liver injury in rats exposed to HS. Results establish that oxidative stress in liver tissue along with LFT parameters of plasma were increased with rise in T_c. But, it was captivating to witness that this pattern was not followed in LFT of tissue as well as in expression level of cytoprotective and apoptotic proteins. Surprisingly, these constraints declined in SHS with respect to MHS rats but remained higher as compared to control. The possible reason for this could be the alteration in protein structure and synthesis which is not maintained due to HS and heat stroke (increased T_a and T_c). Results of histological study also reveal that there is a significant loss of parenchymal tissue with distortion in lobular orientation. Thus, increased levels of LFT and oxidative stress parameters is in equivalent to HE stained tissue sections pattern resulting perturbation in liver parenchyma in HS rats.

REFERENCES

1. Bouchama, A. & Knochel, J.P. Heat stroke. *N. Engl. J. Med.,* 2002, **346**(25), 1978–1988. doi: 10.1056/NEJMra011089.
2. Glazer, J.L. Management of heatstroke and heat exhaustion. *Am. Fam. Physician.*, 2005, **71**(11), 2133–2140. PMID: 15952443.
3. Hsieh, Y.C.; Chen, R.F.; Yeh, Y.S.; Lin, M.T.; Hsieh, J. H. & Chen, S.H. Kynurenic acid attenuates multiorgan dysfunction in rats after heat stroke. *Acta. Pharmacol. Sin.,* 2011, **32**(2), 167–174. doi: 10.1038/aps.2010.191.
4. Varghese, G.M.; John, G.; Thomas, K.; Abraham, O.C. & Mathai, D. Predictors of multi-organ dysfunction in heat stroke. *Emerg. Med. J.*, 2005, **22**(3), 185–187. doi: 10.1136/emj.2003.009365.
5. Liu, C. C.; Shih, M.F.; Wen, Y.S.; Lai, Y.H. & Yang, T.H. Dexamethasone improves heat stroke-induced multiorgan dysfunction and damage in rats. *Int. J. Mol. Sci.*, 2014, **15**(11), 21299–21313. doi: 10.3390/ijms151121299.
6. Leon, L.R. & Bouchama, A. Heat stroke. *Compr. Physiol.*, 2015, **5**(2), 611–647. doi: 10.1002/cphy.c140017.
7. Epstein, Y. & Moran, D.S. Thermal comfort and the heat stress indices. *Ind. Health,* 2006, **44**(3), 388–398. doi: 10.2486/indhealth.44.388.
8. Reule, S. & Drawz, P.E. Heart rate and blood pressure: Any possible implications for management of hypertension? *Curr. Hypertens. Rep.,* 2012, **14**(6), 478–484. doi: 10.1007/s11906-012-0306-3.
9. Corrêa, T.D.; Vuda, M.; Takala, J.; Djafarzadeh, S.; Silva, E. & Jakob, S.M. Increasing mean arterial blood pressure in sepsis: Effects on fluid balance, vasopressor load and renal function. *Crit. Care.,* 2013, **17**(1), R21. doi: 10.1186/cc12495.
10. Bourgoin, A.; Leone, M.; Delmas, A.; Garnier, F.; Albanèse, J. & Martin, C. Increasing mean arterial pressure in patients with septic shock: Effects on oxygen variables and renal function. *Crit. Care Med.,* 2005, **33**(4), 780–786. doi: 10.1097/01.CCM.0000157788.20591.23.
11. Kew, M.; Bersohn, I.; Seftel, H. & Kent, G. Liver damage in heat stroke. *Am. J. Med.,* 1970, **49**(2), 192–202. PMID: 5452941.
12. Blann, A. What is the purpose of liver function tests? *Nurs. Times,* 2014, **110(**6), 17–19. PMID: 24669469.
13. Singh, A.; Bhat, T.K.; & Sharma, O.P. Clinical biochemistry of hepatotoxicity. *J. Clin. Toxicol.,* 2011, S**4**. doi: 10.4172/2161-0495.S4-001.
14. Li, D.; Wang, X.; Liu, B.; Liu, Y.; Zeng, Z.; Lu, L.; Zheng, Z.; Li, B. & Zheng, Z. Exercises in hot and humid environment caused liver injury in a rat model. *PloS One,* 2014, **9**(12), e111741. doi: 10.1371/journal.pone.0111741.eCollection 2014.
15. Sreedhar, A.S. & Csermely, P. Heat shock proteins in the regulation of apoptosis: New strategies in tumor therapy. *Pharmacol. Ther.,* 2004, **101**(3), 227–257. doi: 10.1016/j.pharmthera.2003.11.004.
16. Ippolito, D.L.; Lewis, J.A.; Yu, C.; Leon, L.R. & Stallings, J.D. Alteration in circulating metabolites during and after heat stress in the conscious rat: potential biomarkers of exposure and organ-specific injury. *BMC Physiol.,* 2014,

14(1), 1–17.
doi: 12899-014-0014-0.

17. Li, T.; Ding, F.; Sun, Q.; Zhang, Y. & Kinney, P.L. Heat stroke internet searches can be a new heat wave health warning surveillance indicator. *Sci. Rep.,* 2016, **6**, 37294. doi: 10.1038/srep37294.
18. Hall, P. & Cash, J. What is the real function of the liver 'function' tests? *Ulster Med. J.,* 2012, **81**(1), 30–36. PMID: 23536736.
19. Levick, C. How to interpret liver function tests. *South Sudan Med. J.*, 2017, **10**(2), 40-43. http://www.southsudanmedicaljournal.com/archive/may-2017/how-to-interpret-liver-function-tests.html.
20. Akbarian, A.; Michiels, J.; Degroote, J.; Majdeddin, M.; Golian, A. & De Smet, S. Association between heat stress and oxidative stress in poultry; mitochondrial dysfunction and dietary interventions with phytochemicals. *J. Anim. Sci. Biotechnol.,* 2016, **7**, 37. doi: 10.1186/s40104-016-0097-5, eCollection 2016.
21. Slimen, I.B.; Najar, T.; Ghram, A.; Dabbebi, H.; Ben Mrad, M. & Abdrabbah, M. Reactive oxygen species, heat stress and oxidative-induced mitochondrial damage. A Review. *Int. J. Hyperthermia,* 2014, **30(**7), 513–523. doi: 10.3109/02656736.2014.971446.
22. Kregel, K.C. & Zhang, H.J. An integrated view of oxidative stress in aging: basic mechanisms, functional effects, and pathological considerations. *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, 2007, **292**(1), R18-36. doi: 10.1152/ajpregu.00327.2006.
23. Chakraborty, H.; Ray, S.N. & Chakrabarti, S. Lipid peroxidation associated protein damage in rat brain crude synaptosomal fraction mediated by iron and ascorbate. *Neurochem. Int.*, 2001, **39**(4), 311–317. doi: 10.1016/S0197-0186(00)00117-0.
24. Tell, G.; Vascotto, C. & Tiribelli, C. Alterations in the redox state and liver damage: Hints from the EASL basic school of hepatology. *J. Hepatol.*, 2013, **58(**2), 365–374. doi: 10.1016/j.jhep.2012.09.018.
25. Mayer, M.P. & Bukau, B. Hsp70 Chaperones: Cellular functions and molecular mechanism. *Cell. Mol. Life Sci.*, 2005, **62**(6), 670–684. doi: 10.1007/s00018-004-4464-6.
26. Calderwood, S.K.; Khaleque, M.A.; Sawyer, D.B. & Ciocca, D.R. Heat shock proteins in cancer: Chaperones of tumorigenesis. *Trends Biochem. Sci.*, 2006, **31**(3), 164–172. doi: 10.1016/j.tibs.2006.01.006.
27. Lanneau, D.; de Thonel, A.; Maurel, S.; Didelot, C. & Garrido, C. Apoptosis versus cell differentiation: Role of heat shock proteins HSP90, HSP70 and HSP27. *Prion*, 2007, **1**(1), 53–60. PMCID: PMID: 19164900 .
28. Fulda, S.; Gorman, A.M.; Hori, O. & Samali, A. Cellular stress responses: Cell survival and cell death. *Int. J. Cell. Biol.*, 2010, **2010**, 1-23. doi: 10.1155/2010/214074.
29. Li, C.Y.; Lee, J.S.; Ko, Y.G.; Kim, J.I. & Seo, J.S. Heat shock protein 70 inhibits apoptosis downstream of cytochrome c release and upstream of caspase-3 activation. *J. Biol. Chem.*, 2000, **275**(33), 25665–25671. doi: 10.1074/jbc.M906383199.
30. Zhang, H.J.; Xu, L.; Drake, V.J.; Xie, L.; Oberley, L.W. & Kregel, K.C. Heat-induced liver injury in old rats is associated with exaggerated oxidative stress and altered transcription factor activation. *FASEB J.*, 2003, **17(**15), 2293–2295. doi: 10.1096/fj.03-0139fje.
31. Xing, H.; Jia, K.; He, J.; Shi, C.; Fang, M.; Song, L.; Zhang, P.; Zhao, Y.; Fu, J. & Li, S. Establishment of the tree shrew as an alcohol-induced fatty liver model for the study of alcoholic liver diseases. *PLoS One*, 2015, **10**(6), e0128253. doi: 10.1371/journal.pone.0128253.
32. Chauhan, N.R.; Kapoor, M.; Prabha Singh, L.; Gupta, R.K.; Meena, R.C.; Tulsawani, R.; Nanda, S. & Singh, S.B. Heat stress-induced neuroinflammation and aberration in monoamine levels in hypothalamus are associated with temperature dysregulation. *Neuroscience*, 2017, **358**, 79–92. doi: 10.1016/j.neuroscience.2017.06.023.
33. Geng, Y.; Peng, N.; Liu, Y.N.; Li, X.G.; Li, B.L.; Peng, L.Q.; Ma, Q. & Su, L. Physical effort affects heat stroke thermoregulatory response and mortality in rats. *Shock,* 2015, **44**(2), 149–156. doi: 10.1097/SHK.0000000000000387.
34. Hubbard, R.W.; Matthew, W.T.; Linduska, J.D.; Curtis, F.C.; Bowers, W.D.; Leav, I. & Mager, M. The laboratory rat as a model for hyperthermic syndromes in humans. *Am. J. Physiol.*, 1976, **231**(4), 1119–1123. doi: 10.1152/ajplegacy.1976.231.4.1119.
35. Bae, D.D.; Brown, P.L. & Kiyatkin, E.A. Procedure of rectal temperature measurement affects brain, muscle, skin, and body temperatures and modulates the effects of intravenous cocaine. *Brain Res.*, 2007, **1154,** 61–70. doi: 10.1016/j.brainres.2007.03.078.
36. Fischer, A.H.; Jacobson, K.A.; Rose, J. & Zeller, R. Hematoxylin and eosin staining of tissue and cell sections. *CSH Protoc.*, 2008, **2008,** pdb. prot4986. doi: 10.1101/pdb.prot4986.
37. Hota, S.K.; Barhwal, K.; Baitharu, I.; Prasad, D.; Singh, S.B. & Ilavazhagan, G. Bacopa monniera leaf extract ameliorates hypobaric hypoxia induced spatial memory impairment. *Neurobiol. Dis.*, 2009, **34**(1), 23–39. doi: 10.1016/j.nbd.2008.12.006.
38. Lebel, C.P. & Bondy, S.C. Sensitive and rapid quantitation of oxygen reactive species formation in rat synaptosomes. *Neurochem. Int.*, 1990, **17**(3), 435–440. PMID: 20504643.
39. Levine, R.L.; Garland, D.; Oliver, C.N.; Amici, A.; Climent, I.; Lenz, A.G.; Ahn, B.W.; Shaltiel, S. & Stadtman, E.R. Determination of carbonyl content in oxidatively modified proteins. *Methods Enzymol.*, 1990, **186**, 464–478. PMID: 1978225.
40. Floor, E. & Wetzel, M.G. Increased protein oxidation in human substantia nigra pars compacta in comparison with basal ganglia and prefrontal cortex measured with an improved dinitrophenylhydrazine assay. *J. Neurochem.*,

1998, **70**(1), 268–275. PMID: 9422371.
41. Wehrwein, E.A. & Joyner, M.J. Regulation of blood pressure by the arterial baroreflex and autonomic nervous system. *Handb. Clin. Neurol.*, 2013, **117**, 89–102. doi: 10.1016/B978-0-444-53491-0.00008-0.
42. Ogoh, S.; Sato, K.; Okazaki, K.; Miyamoto, T.; Hirasawa, A.; Morimoto, K. & Shibasaki, M. Blood flow distribution during heat stress: Cerebral and systemic blood flow. *J. Cereb. Blood Flow Metab.*, 2013, **33**(12), 1915–1920. doi: 10.1038/jcbfm.2013.149.
43. Lee, C.W.; Perng, C.L.; Huang, Y.S.; Luo, J.C.; Hung, C.L. & Lin, H.C. Multiple organ failure caused by non-exertional heat stroke after bathing in a hot spring. *J. Chin. Med. Assoc.,* 2010, **73**(4), 212–215. doi: 10.1016/S1726-4901(10)70044-7.
44. Lumlertgul, D.; Chuaychoo, B.; Thitiarchakul, S.; Srimahachota, S.; Sangchun, K. & Keoplung, M. Heat stroke-induced multiple organ failure. *Ren. Fail.*, 1992, **14**(1), 77–80. PMID: 1561392.
45. Adeyemi1, O.T.; Osilesi, O.; Adebawo1, O.O.; Onajobi, F.D.; Oyedemi, S.O. & Afolayan, A.J. Alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities in selected tissues of rats fed on processed atlantic horse mackerel (Trachurus trachurus). *Adv Biosci Biotech*, 2015, **6**, 139-152. doi: 10.4236/abb.2015.63014.
46. Vítek, L. The role of bilirubin in diabetes, metabolic syndrome, and cardiovascular diseases. *Front. Pharmacol.*, 2012, **3**, 55. doi: 10.3389/fphar.2012.00055.
47. Yakubu, M.T.; Bilbis, L.S.; Lawal, M. & Akanji, M.A. Evaluation of selected parameters of rat liver and kidney function following repeated administration of yohimbine. *Biokemistri*, 2003, **15(**2), 50-56. https://www.ajol.info/index.php/biokem/article/view/136133.
48. Goto, A.; Egawa, T.; Sakon, I.; Oshima, R.; Ito, K.; Serizawa, Y.; Sekine, K.; Tsuda, S.; Goto, K. & Hayashi, T. Heat stress acutely activates insulin-independent glucose transport and 5'-AMP-activated protein kinase prior to an increase in HSP72 protein in rat skeletal muscle. *Physiol. Rep.*, 2015, **3**(11), e12601. doi: 10.14814/phy2.12601.
49. Valvona, C. J.; Fillmore, H.L.; Nunn, P.B. & Pilkington, G.J. The regulation and function of lactate dehydrogenase A: Therapeutic potential in brain tumor. *Brain Pathol.*, 2016, **26**(1), 3–17. doi: 10.1111/bpa.12299.
50. Li, S.; Tan, H.Y.; Wang, N.; Zhang, Z.J.; Lao, L.; Wong, C.W. & Feng, Y. The role of oxidative stress and antioxidants in liver diseases. *Int. J. Mol. Sci.*, 2015, **16**(11), 26087–26124. doi: 10.3390/ijms161125942.
51. Kattaia, A.A.A.; Abd El-Baset, S.A.; Mohamed, E.M.; Abdul-Maksou, R.S. & Elfakharany, Y.M. Molecular mechanisms underlying histological and biochemical changes induced by nitrate in rat liver and the efficacy of S-Allylcysteine. *Ultrastruct. Pathol.*, 2017, **41**(1), 10–22. doi: 10.1080/01913123.2016.1252821.
52. Ayala, A.; Muñoz, M.F. & Argüelles, S. Lipid peroxidation: Production, metabolism, and signaling mechanisms of malondialdehyde and 4-Hydroxy-2-Nonenal. *Oxid. Med. Cell. Longev.*, 2014, **2014**, 360438. doi: 10.1155/2014/360438.
53. Ilievska, J.; Cicimov, V.; Antova, E.; Gjorgoski, I.; Hadzy-Petrushev, N. & Mladenov, M. Heat-induced oxidative stress and inflammation in rats in relation to age. *Res Phy Edu Sport & Health*, 2016, **5**(2), 123-130.
54. Jaeschke, H. & Woolbright, B.L. Current strategies to minimize hepatic ischemia-reperfusion injury by targeting reactive oxygen species. *Transplant. Rev.*, 2012, **26**(2), 103–114. doi: 10.1016/j.trre.2011.10.006.
55. Oosten-Hawle, P.V.; Porter, R.S. & Morimoto, R.I. Regulation of organismal proteostasis by transcellular chaperone signaling. *Cell*, 2013, **153**(6), 1366–1378. doi: 10.1016/j.cell.2013.05.015.
56. Pockley, A.G. Heat shock proteins in health and disease: Therapeutic targets or therapeutic agents? *Expert Rev. Mol. Med.*, 2001, **3**(23), 1–21. doi: 10.1017/S1462399401003556.
57. Wu, C. Heat shock transcription factors: Structure and regulation. *Annu. Rev. Cell Dev. Biol.*, 1995, **11**, 441–469. doi: 10.1146/annurev.cb.11.110195.002301.
58. Dehbi, M.; Baturcam, E.; Eldali, A.; Ahmed, M.; Kwaasi, A.; Chishti, M.A. & Bouchama, A. Hsp-72, a candidate prognostic indicator of heat stroke. *Cell Stress Chaperones*, 2010, **15**(5), 593–603. doi: 10.1007/s12192-010-0172-3.

CONFLICT OF INTERESTS

The authors declared that there is no conflict of interest.

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