# Hepatic Expression Level of HSP70 in Thymoquinone-Treated Heat-Stressed Male Rats

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## Abstract

The Present Study Has Been Conducted To Investigate The Role Of Thymoquinone In Elucidation Of Hepatic Tissue To Overproduce Heat Shock Protein 70 Gene Under Chronic Heat Stress In Mature Male Rats. Ninety Mature Sprague Dewily Male Rats Were Randomly Assigned To Three Equal Groups,Treated For 3 Weeks As Follow: Non-Stressed Male Rats (Group C) Have Been Kept At Normal Room Temperature (20 ±1Cº) And Daily Supplemented With Distilled Water, Heat-Stressed Male Rats (Groups HS And HSTQ)Have Been Kept At High Room Temperature (35±1Cº) For 6 Hours A Day, And Daily Administered With Distilled Water And TQ Suspension (50 Mg/Kg Bw, Po), Respectively. At The End Of Each Week, 10 Males From Each Group Were Anaesthetized With Thiopental (100 Mg/ Kg, I.P.), Sacrificed And Livers Were Removed. Samples From Hepatic Tissues Have Been Quickly Dipped In DEPC Solution, And Frozen At -80 ºc For Determination Of Hsp70 Gene Expression Levels By Semi-Quantitative RT-PCR Analyses.The Results Of Hsp70 Gene Mrna Registered Increased Expression Levels Of Heat Stressed Males (HS And HSTQ Groups) Compared With Control Males At The 2nd Week Of Experiment, Which Continued In HSTQ Group At The 3rd Week Of Experiment, But HS Group Showed Significant Decline To Subnormal Levels That Recorded By Control.In Conclusion It Can Be Proposed That TQ Supplementation Can Be Used As A Potent Therapeutic Agent To Combat Heat Stress.

**KEYWORD:** Inhibin, Thymoquinone, Heat Stress, Hsp70 Gene, Liver

## Introduction

In The Last Years, We Are Facing Extremes Of High Ambient Temperatures Which Affecting The Feature Of Life And Continuity Of Life Style Regarding To The Physical Efficiency Of The Individuals. To Meet This Challenge, There Is A Need To Combat The Morbidity And Mortality Associated With Heat Stress (Jan Et Al., 2015). Several Studies Have Shown That Heat Stress Increases The Protein Unfolding By The Help Of Other Proteins Called Chaperones. HSP70 Is One Of These Chaperones, Where Its Levels Have Already Been Associated With Heat Stress In Humans (Mosser Et Al., 1997; Feder And Hofmann, 1999; Kregel, 2002; Marini Et Al., 2007; Tuttle Et Al., 2015), Rats (Arnaud Et Al., 2002), Cattle (Gaughan Et Al., 2013), Plants (Kregel, 2002). Therefore, The Chaperones Can Be Used As A General Indicator Of Different Stresses But Not The Only Biomarker For Heat Stress. There Is Need To Explore Other Biomarkers Which Can Specifically Mark Heat Stress And Acquisition Of Acclimation Along With Chaperones (Jan Et Al., 2015). The Hsps Are Activated In Response To Stressors (Iwaki Et Al., 1993). The HSP Act In Cellular And Tissue Homeostasis (Thompson Et Al., 2002; De Jong Et Al., 2009) And Are Released Intracellularly And Extracellularly In An

Inducible Form In Response To Stress (Hightower And Guidon, 1989; Hecker And Mcgarvey, 2011). Among The HSP, HSP70 Has A Significant Role In Cell Thermotolerance (King Et Al., 2002; Beckham Et Al., 2004).

Thymoquinone, A Potent Constituent Which Has Been Obtained From *Nigella Sativa* Seeds (Gilani, 2004), Have Been Employed For Many Years For Improvement Of Lipid Profile (Zaoui*et Al*., 2002; Kalus*et Al.*, 2003; Al-Sa'aidi Et Al., 2015), Hyperglycemia Of Diabetic Rats (Al-Sa'aidi Et Al., 2014), Immune Modulation (Kalus*et Al*., 2003; Hamady, 2011), And Possess Antioxidant Effects Through Enhancing The Oxidant Scavenger System As Well As Its Potent Antiinflammatory Activity (Salem, 2005).

The Present Study Has Been Carried Out To Explore The Possible Role Of Thymoquinone Supplementation In Ameliorating The Side Effects Of Heat Stress By Evaluating The Expression Level Of Hsp70 Gene In Hepatic Tissues Of Heat Stressed Male Rats.

**Materials And Methods**

**Experimental Animals:**Adult Sprague Dewily Male Rats (Average Weight Was 232 ± 8.56g.), Were Used In The Present Study. The Animals Were Housed Under Laboratory Conditions (12L:12D Cycles At 20-22 C°) And Fed Standard Laboratory Food (19% Protein Ratio And 3000 Kilocalories Energy) And Drinking Water*ad Libitum*.

**Preparation Of TQ Suspension:** TQ Has Been Provided By Sigma Aldrich, UK And Used At A Dose Of 50 Mg Of TQ Suspension/Kg Bw (Kanter, 2009). The Suspension Was Prepared By Dissolving 5 Mg Of TQ Powder In 1 Ml Of Distilled Water To Be Used As 5 Mg/1 Ml/ 100 G Bw (For HSTQ Group).

**Heat Stress Protocol:** According To Sutherland Et Al. (2006), The Rats Have Been Kept, During The First Few Days, At Normal Room Temperature (21±1Cº). During Experimental Periods (3 Weeks), Heat Stressed Groups Have Been Exposed To High Ambient Temperature (35±1 °C) For 6 Hours Aday.

**Experimental Design:** Ninety Male Rats Were Randomly Assigned To Three Equal Groups, Treated For 3 Weeks As Follow: Non-Stressed Male Rats (Groupc) Have Been Kept At Normal Room Temperature (20 ±1Cº) And Daily Supplemented With Distilled Water, Heat-Stressed Male Rats (Groups HS And HSTQ)Have Been Kept At High Room Temperature (35±1Cº) For 6 Hours A Day, And Daily Administered With Distilled Water And TQ Suspension (50 Mg/Kg Bw, Po), Respectively. At The End Of Each Week, 10 Males From Each Group Were Anaesthetized With Thiopental (100 Mg/ Kg, I.P.), Sacrificed And Livers Were Removed. Samples From Hepatic Tissues Have Been Quickly Dipped In DEPC Solution, And Frozen At -80 ºc For Determination Of Hsp70 Gene Expression Levels By Semi-Quantitative RT-PCR Analyses.

**RNA Extraction:** RNA Has Been Extracted From The Hepatic Tissues According To Surzcki (2000).

**Polymerase Chain Reaction (PCR):** Master Mix For Each Sample Was Prepared According To The Recommendations Of The Manufacturers, And The Same Procedure Followed In The Determination Of Endogenous Gene And Target Genes.

**Gel Electrophoresis:** PCR Products Have Been Transferred Into Gel Electrophoresis Apparatus For Obtaining And Determining The Studied Genes Bands. This Step Has

Been Done By Mixing 2 µl Of Loading Dye With 10 µl Of Each PCR Product (Each Sample) And 2 µl Of Ladder. The Mixture Was Loaded Into Gel Lanes. Finally, Gel Electrophoresis Apparatus Was Turned On At 90 Mv For 40 Minutes.

**Gel Documentation:** Gel Documentation Step Has Been Carried Out According To Surzcki (2000).

**Statistical Analysis**: The Results Were Expressed As Mean ± Standard Deviation Of The Mean (SDM). Comparisons Were Performed Using One Way Analysis Of Variance (ANOVA1) And Newman- Keuls To Test All Groups' Unpaired Values. Differences Were Considered To Be Significant At The Level Of $P<0.05$. The Statistical Analysis Was Carried Out Using The Graphpad Prism (SAS Institute, Inc., USA, 2011).

**Results**

**Concentration Of RNA In The Testis**: The Result Of RNA Concentration In Hepatic Tissues From HSTQ Group Recorded Significant Elevation ($P\leq 0.05$), At 2nd And 3rd Weeks, Compared With Control And HS Groups, Whereas HS Group Increased Significantly ($P\leq 0.05$) Than Control At The 2nd Week, But It Declined At The 3rd Week (Figure 1).

**RNA Normalization:**The Present Findings Illustrates Ratio Within Normal Range (More Than 1.8 And Less Than 2.1) Between The Optical Densities At 260 Nm And 280 Nm For Each Sample As Well As The Dilution Folds Needed To Prepare The Final Concentration (100 Ng/µl) Necessary To Complete The Following Steps Of Semi-Quantitative RT-PCR.

**Mrna Expression Level Of Hsp70 Gene In Hepatic Tissues:** The Results Of Mrna Expression Level Of Hsp70 Gene, Illustrated In Figures (2), Registered Increased Expression Levels Of Heat Stressed Males (HS And HSTQ Groups) Compared With Control Males At The 2nd Week Of Experiment ($P\leq 0.05$). This Significant Elevation Continued In HSTQ Group At The 3rd Week Of Experiment, But HS Group Showed Significant Decline To Subnormal Levels That Recorded By Control.

**Discussion**

The Up-Regulation Of HSP70 Expression Levels In Liver Tissues During High Ambient Temperature Due To Administration Of TQ Suspensioncould Be Resulted In Significant Increase Of Soluble HSP70 In The Blood Plasma And In The Brain (Fehrenbach*et Al.*, 2004). This Released Extracellular HSP70 May Play Key Role In Initiating Immunoregulatory Functions. Therefore, It Can Be Speculated That Elevated Intra- And Extracellular HSP70 Levels After TQ Administration, During Heat Stress, Might Act As Beneficial Danger Signals Protecting Against Stressful Condition Via Cytokines Secretion (Campisi*et Al.*, 2003). This Hypothesis Might Be Supported By The Finding That Treatment With *N. Sativa* Seed Extract, Under Normal And High Ambient Temperature, Also Results In An Increase Of Pro-Inflammatory (TNF-A Activity) And Anti-Inflammatory (IL-4 Activity) Cytokines (Hamady, 2011).These Findings Might Be Considered As Indicative To The Potent Role Of TQ In The Activation Of The Innate Response And Activation Of The Cellular Arm Of The Adaptive Immunity In Mature Male Rats, When Used At The Given Doses For Three Weeks.

From The Present Findings, It Has Been Found That Expression Levels Of Heat Shock Protein 70 Was Positively Correlated To High Ambient High Ambient Temperature And Heat Stress Period Dependent As Well, Since It Was Concurrent With The Expanding Of Stress Period. This Finding Was In Agreement With That Observed By Gaughan Et Al. (2013) Who Suggested That The HSP70 Concentration Is A Reliable Indicator Of Chronic Stress.

It Has Been Shown, In The Present Study, That Exposure Of Male Rats Heat Stress Challenge, The HSP70 Response Is Elicited During A Week. Studies With Rats (Sareh Et Al., 2011) And Humans (Thompson Et Al 2002; Ogura Et Al., 2008; Hom Et Al., 2012) Reported A Reduction In HSP70 Expression When Subjects Were Exposed To Repeated Bouts Of Exercise-Induced Heat Stress. In The Present Study, The Reduction Of HSP70 Has Been Shown After Two Weeks Of Exposure To The Heat Stress. This Reduction May Be Due To Acclimation To The Stressor. This May Explain The Reduction In HSP70 After Two To Three Weeks That Was Seen In The Current Study. It Has Been Found That Exposure To Heat Stress Will Induce A HSP Response Resulting In Intracellular Concentrations Of HSP70 As Well As Other HSP (Feder And Hofmann, 1999; Johnson Et Al., 2005; Collier Et Al., 2006). Extracellular And Intracellular HSP70 Has Been Found In Elevated Levels In Stressed And Non-Stressed Humans (Aneja Et Al., 2006; Park Et Al., 2006), Rats (Johnson Et Al., 2005), And Cattle (Gaughan Et Al., 2013). It Is Possible That Damaged Cells, Due To The Animal Being Exposed To Prolonged Heat Stress, May Be The Causal Factor Leading To HSP70 Expression (Lambert, 2009).

As Well As To Its Oxidants Scavenging Activity (Salem, 2005), TQ Is Considered As A Good Antioxidant Through Decreasing The Stress Hormones Such As Cortisol (Hamady, 2011), Anti-Hypolipidemic Agent Through Increasing HDL (The Healthy Cholesterol) And Decreasing LDL And Triglycerides (Zaoui*et Al*., 2002; Kalus*et Al.,* 2003; Al-Sa'aidi Et Al., 2015) And Anti-Hypoglycemic Agent In Diabetic Animals Through Decreasing Blood Glucose Levels (Al-Sa'aidi Et Al., 2014), Decreasing Hepatic Gluconeogenesis (Farah Et Al., 2005) And Enhancing Of Insulin Secretion (Farah Et Al., 2002; Alsa'aidi Et Al., 2014b), As Well As Its Potent Immune Modulation (Kalus*et Al*., 2003; Salem, 2005; Hamady, 2011).

Results From The Present Study Provided Important Information Concerning The Possible Therapeutic Use Of Thymoquinone Improving Heat Stress Side Effects In Mammals. In Conclusion It Can Be Proposed That TQ Supplementation Can Be Used To Combat Heat Stress, Since It Will Provide A Potent Antioxidant Activity, Particularly During Summer.

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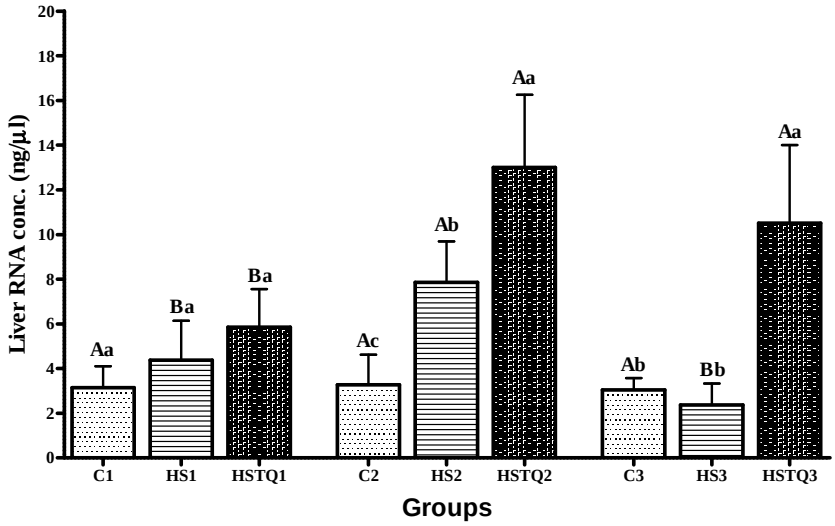


**Figure (1): Effect Of TQ On RNA Concentration (Ng/µl) In Liver Tissues Of Heat-Stressed Adult Male Rats.**

The Results Represented As Mean ± Standard Deviation Of The Mean.

Different Small Letters Denotes The Present Of Significant Differences ($P<0.05$) Between Groups For Each Period.

Different Capital Letters Denotes The Present Of Significant Differences ($P<0.05$) Between Periods For Each Group.

**C**: Intact Male Rats Kept At Normal Temperature (20 ±1Cº) And Supplemented With Distilled Water For 3 Weeks.**HS**: Heat-Stressed Male Rats Kept At High Temperature (35 ±1Cº) For 6 Hours A Day And Supplemented With Distilled Water For 3 Weeks.**HSTQ**: Heat-Stressed Male Rats Kept At High Temperature (35 ±1Cº) For 6 Hours A Day And Supplemented With TQ Suspension (50 Mg/Kg Bw, Po) For 3 Weeks.

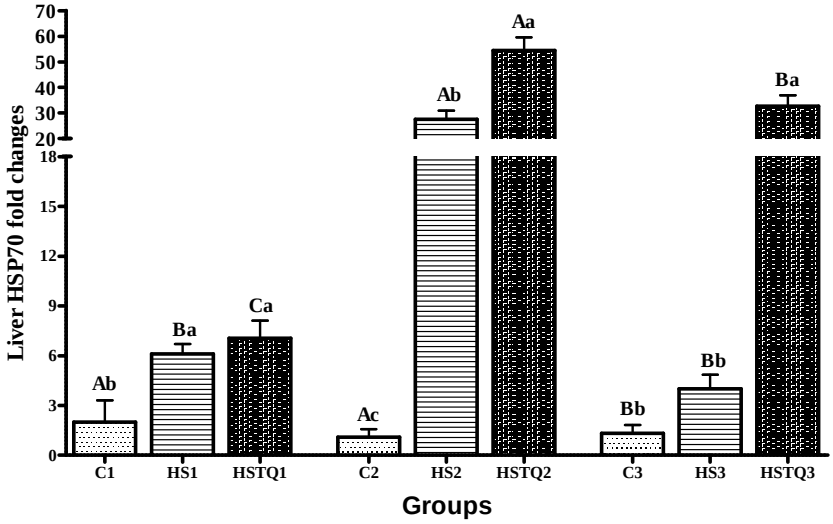


**Figure (2): Effect Of TQ On Liver Hsp70 Fold Changes Of Heat- Stressed Adult Male Rats.**

The Results Represented As Mean ± Standard Deviation Of The Mean.

Different Small Letters Denotes The Present Of Significant Differences ($P<0.05$) Between Groups For Each Period.

Different Capital Letters Denotes The Present Of Significant Differences ($P<0.05$) Between Periods For Each Group.

**C**: Intact Male Rats Kept At Normal Temperature (20 ±1C°) And Supplemented With Distilled Water For 3 Weeks. **HS**: Heat-Stressed Male Rats Kept At High Temperature (35 ±1C°) For 6 Hours A Day And Supplemented With Distilled Water For 3 Weeks. **HSTQ**: Heat-Stressed Male Rats Kept At High Temperature (35 ±1C°) For 6 Hours A Day And Supplemented With TQ Suspension (50 Mg/Kg Bw, Po) For 3 Weeks.