Theantioxidant potent of Thymoquinone (TQ) against heat stress in male rats

[a]Jabbar A.A. Alsaadi, [b]Hussein K.O. Al-Mayali, [c]Najlaa O.H. Al-Jubury,

[a]BVMS, MS, PhD; Professor, Physiology, Dept. Physiol. & Pharmacol., College of Vet. Med., Al-QadisiyaUniv, Iraq.

[b]BS, MS, PhD; Asst. Prof., Physiology, Dept. Biology, College of Education, Al-QadisiyaUniv, Iraq.

[c]BS, MS, PhD student; Lect., Dept. Biology, College of Education, Al-QadisiyaUniv, Iraq.

Abstract

To examine the role of thymoquinone (TQ), the active compound of *Nigella sativa* seeds, in ameliorating the oxidative effects of chronic heat stress, two hundred and forty male rates were randomly assigned to four equal groups daily administered for 42 days with; distal water under normal ambient temperature (22-25 ºC) (intact males; control group), distal water under high ambient temperature (35-40 ºC) (heat stressed males; HS group), TQ suspension (50 mg/kg, bw) under high ambient temperature (35-40 ºC) (heat stressed males treated with TQ; HSTQ group), and TQ suspension (50 mg/kg, bw) under normal ambient temperature (22-25 ºC) (intact males treated with TQ; TQ group). At the end of treatment period, male rats were anesthetized by single injection of thiopental (100 mg/ kg, i.p.), sacrificed, and blood samples were obtained from abdominal vein for assessment of oxidants and antioxidants concentrations. Male rats that have been reared under high ambient temperature without treatment (HS group) registered marked increase in serum concentrations of malondialdehyde, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, superoxide dismutase, catalase as well as activities of glutathione peroxidase, glutathione transferase, and marked decrease in reduced glutathione concentration and glutathione reductase activity, whereas those treated with TQ (HSTQ group) showed slight increase. It can be concluded that TQ potentially induce endogenous enzymatic antioxidants under high ambient temperature in male rats.

KEYWORDS: thymoquinone, *Nigella sativa*, heat stress, antioxidants, oxidative stress

INTRODUCTION:

The variety in climatic variables like temperature, mugginess and radiations were perceived as the potential perils in the development and generation of all residential animal species. High encompassing temperature joined by high air stickiness brought about an extra uneasiness and improved the stress level which thusly brought about misery of the physiological and metabolic exercises of this animal (Ganaie et al., 2013). Stress has been characterized by a few laborers. According to Dobson and Smith (2000), it is uncovered by the powerlessness of an animal to adapt up to its environment, a marvel which is frequently reflected in an inability to accomplish hereditary potential. Rosales (1994) characterized stress as the aggregate adverse impact of different elements on wellbeing and execution of animals. Stress speaks to the response of body to jolts that exasperate typical physiological balance or homeostasis, regularly with impeding impacts as appeared by Khansari et al. (1990). As indicated by Stott (1981), stress is the

aftereffect of environmental powers constantly following up on animals which disturb homeostasis bringing about new adjustments that can be adverse or favorable to the animal.

Among the stressors, heat stress has been of significant worry in diminishing animal's efficiency in tropical, sub-tropical and parched ranges (Silanikove et al., 1997). The extent to which an animal opposes change in body temperature fluctuates with various species on account of contrasts in their warmth managing systems (Salah et al., 1995). Under heat stress, various physiological and behavioral reactions shift in force and term in connection to the animal hereditary make-up and environmental elements through the reconciliation of numerous organs and systems, behavioral, endocrine, cardio-respiratory and immunity (Altan et al., 2003). Responses of homeotherms to direct climatic changes are compensatory and are coordinated at reestablishing thermal balance (West et al., 1999).

Oxidative stress results from overproduction of free radicals and reactive oxygen species, and a reduction in antioxidant safeguard (Trevisan et al., 2001;Williams et al., 2002) reported that oxidation is crucial to almost all cells in the body to give vitality to imperative capacities. Around 95 to 98% of the oxygen devoured is lessened to water amid high-impact digestion system, yet the remaining division might be changed over to oxidative by-items - reactive oxygen species, that may harm the DNA of genes and lead to degenerative changes.

One of the primary purposes behind oxidative stress in animals amid summer in tropics is heat stress. Heat stress happens when the center body temperature of a given species surpasses its extent determined for typical action coming about because of an aggregate heat load (interior heat generation and heat picked up from environment) surpassing the limit for heat scattering (Ganaie et al., 2013).

Nigella sativa extract has been appeared to have immunopotentiating, antioxidant (Buirts and Bucar, 2000), antitumoral (Worthen et al., 1998), and antidiabetic (Meral et al., 2001) properties. A large portion of these properties have been credited fundamentally to the quinone constituents of N. sativa, of which thymoquinone is the primary dynamic element of the volatile oil extracted from the its seeds (Aboutabl et al., 1986). Thymoquinone has been appeared to have potent antioxidant properties (Houghton et al., 1995) and to inhibit the expression of inducible NO synthase in rodent macrophages (El-Mahmoudy et al.,2002). The point of the present study was to explore the antioxidant impacts of thymoquinone under high ambient temperature in male rats.

MATERIALS AND METHODS:

Experimental animals: Mature male Sprague-Dawley rats have been allowed one week to acclimatize to the animal house environment before beginning of experiment. Animals were fed on the standard chow and drinking water *ad libitum* throughout the experimental periods. Room temperature was maintained at 22 ± 2°C, the light-dark cycle was on a 12:12 h with light on at 06:00 a.m and off at 06:00 p.m throughout the experimental period.

Preparation of TQ suspension: Suspension of TQ (Sigma Aldrich, UK) at adose of 50 mg/kg bw (Kanter, 2009) was prepared by dissolving 5 mg of TQ powder in 1 ml of distal water to be used as 5 mg/100 g bw, so that each 100g of body weight will need drenching 1 ml of TQ suspension to be contain 5 mg (for TQ and HSTQ groups).

Experimental design: To determine the sequential alterations (each week) of serum oxidants (MDA) and antioxidants (enzymatic; SOD, CAT, Gr, GS-t, G-px; and non-enzymatic; GSH) activity in response to thymoquinone treatment of chronic heat-stressed male rats. The aim of the consequential assessment of these parameters is to determine the time point of thymoquinone effects. Two hundred and forty adult male rats (weighed 138±4.6 g and aged 56 days) have been assigned to 4 experimental groups of 60 animals each, and treated for 6 weeks as follow: control (intact rats; C group); daily administered with distal water orally and reared under normal ambient temperature (22-25 ºC), HS group; heat stressed rats daily administered with distal water orally and reared under high ambient temperature (35-40 ºC), HSTQ group; heat stressed ratsdaily administered with TQ (50 mg/kgbw, po) and reared under high ambient temperature (35-40 ºC), and TQ group; intact rats daily administered with TQ (50 mg/kg bw, po) and reared under and reared under normal ambient temperature (22-25 ºC).Heat stress has been induced by exposure of male rats to high ambient temperature (at 35-40 ºC for 6 hrs a day) for 42 days.Twenty four hours after the last administration of the treatment, the animals have been processed in the similar manners to those in experiment. One week interval,blood samples have been obtained from each male rats for assessment of serum liver functions (ALT, AST, and ALP concentrations, and oxidant- antioxidants activity (SOD, CAT, GSH, GSH-reductase, GTH-transferase, and MDA).

Assessment of ALP, ALT and AST concentration: Assessment has been performed by using the colorimetric method of Reitman and Frankel (1957).

Assessment of reduced GSH: The absorbance of the reduced chromagen was measured at 412 nm and was directly proportional to the GSH concentration (Burtis and Ashwood, 1999).

Assessment of SOD concentration: By using the modified photochemical Nitrobluetetrazolium (NBT) method in utilizing sodium cyanide as peroxidase inhibitor, SOD levels were assessed (Winterbourn et al., 1975).

Assessment of CATconcentration: According to Aebi (1974) and Kakkar et al. (1984), CAT activity was assessed by measuring the degradation rate of H2O2. The rate of disappearance of H2O2 was monitored spectrophotometrically at 230 nm.

Estimation of lipid peroxidation: The level of peroxidation product; Malondialdehyde (MDA) was measured according to Dillard and Kunnert (1982).

Glutathione reductase activity: This was measured by the method of Carlberg and Mannervik (1975).

Glutathione-transferase activity: This was measured by the method of Habig et al. (1974). Protein was estimated by the method of Lowry et al. (1951).

Statistical analysis: Results were expressed as mean ± standard deviation. Comparisons between groups and periods values were performed using one way analysis of variance (ANOVA1) and Newman- Keuls. Differences were considered to be significant at the level of $P<0.05$. Statistical analysis was carried out using the GraphPad Prism (SAS Institute, Inc., USA).

RESULTS:

In heat stressed (HS) groups, the result illustrated in figure (1) recorded significant ($P<0.05$) increase of SOD concentration compared with control, particularly at the last three weeks in comparison with the first three weeks of treatment. On the other hand, stressed male rats treated with TQ showed significant ($P<0.05$) increase in SOD

concentration as compared with control among the experimental periods, and with male rats treated with TQ, only at the first, fourth, fifth, and sixth week periods. TQ groups recorded higher levels of SOD ($P<0.05$) than control at all periods, but still statistically lower than that of HS and HSTQ groups.

At all of the experimental periods, serum CAT concentration of heat stressed male rats showed significant ($p<0.05$) increase in comparison with control and other groups (Figure 2). On the other hand, HSTQ group was significantly ($p<0.05$) higher than control at the first and second weeks of experiment, then it decreased to show insignificant difference ($p>0.05$) in comparison with control and TQ treated males, at the last four weeks. In comparison with control, TQ treated group showed insignificant difference ($p>0.05$) for the whole 6 weeks (figure 2).

Serum MDA concentration of HS group showed significant ($p<0.05$) increase, at all of the experimental periods, in comparison with control and other groups (Figure 3), whereas TQ treated heat stressed rats showed significant decrease ($p<0.05$) as compared with HS groups, at the same time they showed significant increase ($p<0.05$) as compared with control. TQ treated group showed insignificant difference ($P>0.05$) at the six weeks compared with control, but they were significantly lower than HS and HSTQ groups.

Statistic comparisons showed significant increase ($p<0.05$) of ALP concentration in heat stressed male rats compared with control and other groups, at all experimental periods (figure 4). The results also illustrates insignificant difference ($p>0.05$) for TQ administration alone on serum ALP concentration at all the six weeks, whereas HSTQ treated group showed significant decrease ($p<0.05$) than HS groups, but still significantly ($p<0.05$) higher than control and TQ treated groups.

In comparison with control and TQ groups, HS and HSTQ treated groups showed significant increase ($p<0.05$) of serum ALT concentration at all of the experimental periods (figure 5). HSTQ treated group showed significant decrease ($p<0.05$) as compared with HS group, whereas statistical analysis showed insignificant difference ($p>0.05$) between control and TQ groups at all experimental periods.

Heat stressed male rats (HS) and HSTQ treated groups showed significant increase ($p<0.05$) of serum AST concentration at all of the experimental periods in comparison with control and TQ treated groups (Figure 6). In TQ treated male rats, insignificant difference ($p>0.05$) has been registered in comparison with control, except at the third week which was significantly lower than control.

At the first three weeks of the experimental periods, serum GSH concentration of heat stressed male rats showed significant ($p<0.05$) increase in comparison with control and other groups (Figure 7), then gradually decreased at the fourth week of experiment to reach the lowest level at the sixth week. In the opposite manner, non-stressed TQ treated males recorded the highest level at the sixth week, whereas those under heat stress and treated with TQ showed insignificant ($p>0.05$) levels compared with control.

The result of serum activity of GSH peroxidase in HS and HSTQ groups registered significant ($p<0.05$) decrease than control at the first four weeks of experiment and slightly increased at the fifth and sixth weeks, whereas TQ group showed insignificant ($p>0.05$) difference compared with control group (Figure 8).

Serum GSH-r activity of heat stressed male rats (HS and HSTQ groups) showed significant ($p<0.05$) increase in comparison with control and TQ groups, at the first three weeks of the experimental periods, then HS group showed gradual decrease at the fourth

week of experiment to reach the lowest level at the sixth week, whereas HSTQ group remain significantly higher than control. On the other hand, serum GSH-r activity of non-stressed TQ treated males (TQ group) was significantly higher than control at all periods of the experiment (Figure 9).

In heat stressed (HS and HSTQ) groups, the result illustrated in figure (10) registered significant ($P<0.05$) increase of GS-t activity compared with control at all of the experimental periods. On the other hand, stressed male rats treated with TQ (HSTQ group) showed significant ($P<0.05$) decrease in comparison with non-treated stressed males (HS group), but still significantly ($P<0.05$) higher than control and non-stressed TQ treated males.

DISCUSSION:

The impact of TQ on oxidative stress in heat stressed male rats was assessed biochemically. Heat stress in the experimental animal models show high oxidative stress because of cellular impairment, which exhausts the activity of the antioxidant defense system, advancing the production of free radicals (McEwen, 2005), as it has been accounted for that the intemperate accessibility of free radicals, joined by a decrement in the antioxidant activity, prompts cell dysfunction. Likewise, tissue MDA was significantly elevated, with a significant diminishment in the activity of SOD enzyme in comparison with control group, which may represent the tissue harm shown. The expanded lipid peroxidation brought about disturbance of vital lipid-containing membranes, including the envelope of nucleus, ER membranes, and vacuoles, prompting the high apoptosis. It has been appeared, under oxidative stress, that fragmentation of the mitochondria prompted disturbance of the antioxidative component, reflecting a constrained limit of the mitochondria to conquer the oxidative stress. This perception is in concurrence with the present biochemical result of diminished SOD activity in heat stressed group (HS group). Schettler et al. (1994) recommended that the decreased antioxidant ability was because of higher oxygen metabolites, which bring about abatement in the antioxidant system. The observations of the present study are reliable with past studies reported elevated lipid peroxidation and diminished antioxidant catalysts in diabetes mellitus (El-Missiry and El-Gindy, 2000; Al-Sa'aidi et al, 2002; Kanter et al., 2003; 2004)

Administration of TQ, in intact and heat-stressed male rats, brought in a significant bringing down of raised serum MDA levels after one week treatment and an expansion in non-enzymatic antioxidants (GSH) levels after one week and enzymatic antioxidant (SOD, CAT, GSH-r, GS-t, and GSH-px) levels after two to three weeks treatment. It is also important to note that liver function enzymes (ALT, AST, and ALP) levels in TQ treated heat-stressed males diminished significantly contrasted and those in the untreated heat stressed (Group HS) after three weeks. This distinction may show that the reported antioxidant impact is not related straightforwardly only to direct TQ activity and might be intervened by another mechanism such as induction of endogenous antioxidants. Recently, Meddah et al. (2009) demonstrated that the utilization of the aqueous extract of *N. sativa* in diabetic rats kept any significant changes in MDA levels contrasted and the control group at period of evaluation, while SOD levels were reestablished to normal following two weeks treatment. These findings are in concurrence with the outcomes reported in different studies in trial animals that likewise show that *N. sativa* treatment diminishes MDA and SOD levels (Meral et al., 2001; Houcher et al., 2007; Kanter et al.,

2003; 2004). In our study treatment with TQ (groups HSTQ and TQ) for six weeks reestablished ordinary MDA and antioxidants levels, particularly in TQ group. This shows the oxidants-bringing down impact is not only straightforwardly identified with TQ activity.

TQ treatment reestablished tissue SOD levels and suppressed the elevation in tissue lipid peroxidation during the experiment. Abdelmeguid et al. (2010) registered that TQ enhanced a large portion of the dangerous impacts of STZ, which can be considered as a strong model of oxidative stress, on rat pancreatic islets, with normal morphological β-cells in comparison with control group. This may indicate a compensatory component to adjust to metabolic deviation by separating to supply the energy for both the biosynthesis and liberation of insulin and to induce the biosynthesis of SOD as well as other antioxidants to counteract against oxidative stress in b-cells. This result is concurrence with the biochemical observations of non-significant changes in MDA or lipid peroxidation and reclamation of normal SOD activity shown in the present study.

The outcomes accentuate that TQ displayed strong antioxidant properties by repressing lipid peroxidation and elevating SOD activity. Subsequently, it might be concluded that TQ is not just a direct antioxidant but can also induce the endogenous enzymatic and non- enzymatic antioxidants.

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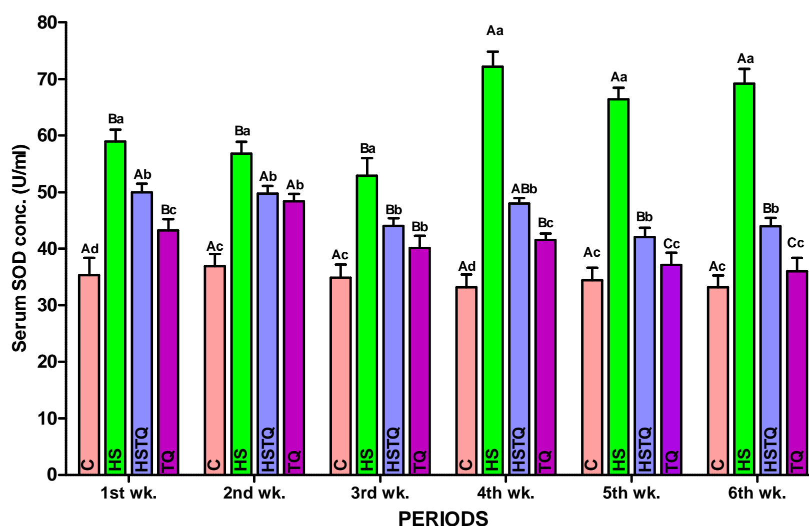

Figure (1): effect of Thymoquinone on serum SOD concentration (U/ml) in heat stressed male rats.

C: intact male rats orally administered with drinking water daily for 42 days and reared under normal ambient temperature (22-25 ºC). **HS:** heat stressed male rats, orally administered with drinking water daily for 42 days and reared under high ambient temperature(35-40 ºC). **HSTQ:** heat stressed male rats, orally administered with TQ suspension (50 mg/kg, bw) daily for 42 days and reared under high ambient temperature(35-40 ºC). **TQ:** intact male rats orally administered with TQ suspension (50 mg/kg, bw) daily for 42 days and reared under normal ambient temperature (22-25 ºC). Data were presented as Mean ±SD of 10 observations (n=10). Different small letters denote significant difference ($p<0.05$) between groups for each period. Different capital letters denote significant difference ($p<0.05$) between periods for each group.

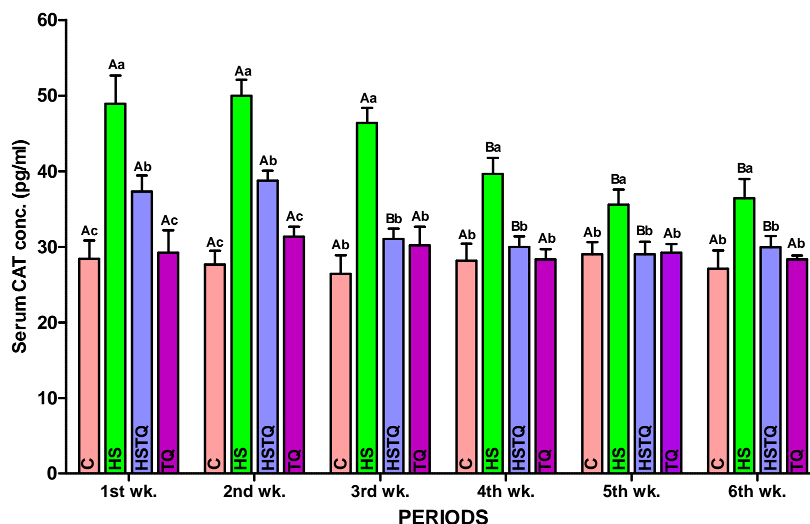

Figure (2): effect of Thymoquinone on serum CAT concentration (U/ml) in heat stressed male rats.

C: intact male rats orally administered with drinking water daily for 42 days and reared under normal ambient temperature (22-25 ºC). **HS:** heat stressed male rats, orally administered with drinking water daily for 42 days and reared under high ambient temperature(35-40 ºC). **HSTQ:** heat stressed male rats, orally administered with TQ suspension (50 mg/kg, bw) daily for 42 days and reared under high ambient temperature(35-40 ºC). **TQ:** intact male rats orally administered with TQ suspension (50 mg/kg, bw) daily for 42 days and reared under normal ambient temperature (22-25 ºC). Data were presented as Mean ±SD of 10 observations (n=10). Different small letters denote significant difference ($p<0.05$) between groups for each period. Different capital letters denote significant difference ($p<0.05$) between periods for each group.

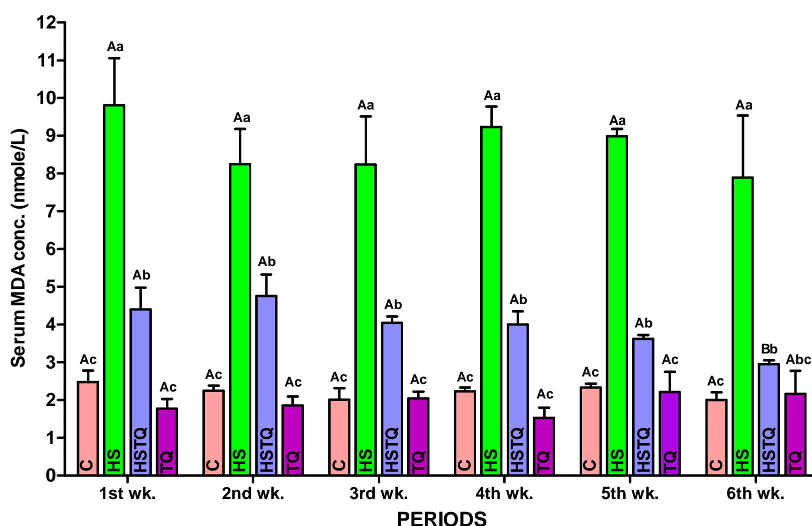

Figure (3): effect of Thymoquinone on serum MDA concentration (nmole/L) in heat stressed male rats.

C: intact male rats orally administered with drinking water daily for 42 days and reared under normal ambient temperature (22-25 ºC). **HS:** heat stressed male rats, orally administered with drinking water daily for 42 days and reared under high ambient temperature(35-40 ºC). **HSTQ:** heat stressed male rats, orally administered with TQ suspension (50 mg/kg, bw) daily for 42 days and reared under high ambient temperature(35-40 ºC). **TQ:** intact male rats orally administered with TQ suspension (50 mg/kg, bw) daily for 42 days and reared under normal ambient temperature (22-25 ºC). Data were presented as Mean ±SD of 10 observations (n=10). Different small letters denote significant difference (p<0.05) between groups for each period. Different capital letters denote significant difference (p<0.05) between periods for each group.

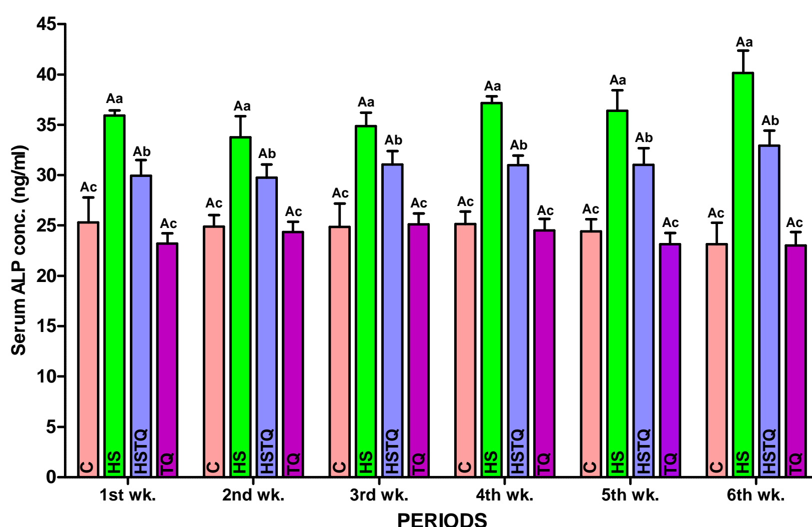

Figure (4): effect of Thymoquinone on serum ALP concentration (ng/ml) in heat stressed male rats.

C: intact male rats orally administered with drinking water daily for 42 days and reared under normal ambient temperature (22-25 ºC). **HS:** heat stressed male rats, orally administered with drinking water daily for 42 days and reared under high ambient temperature(35-40 ºC). **HSTQ:** heat stressed male rats, orally administered with TQ suspension (50 mg/kg, bw) daily for 42 days and reared under high ambient temperature(35-40 ºC). **TQ:** intact male rats orally administered with TQ suspension (50 mg/kg, bw) daily for 42 days and reared under normal ambient temperature (22-25 ºC). Data were presented as Mean ±SD of 10 observations (n=10). Different small letters denote significant difference (p<0.05) between groups for each period. Similar capital letters denote insignificant difference (p>0.05) between periods for each group.

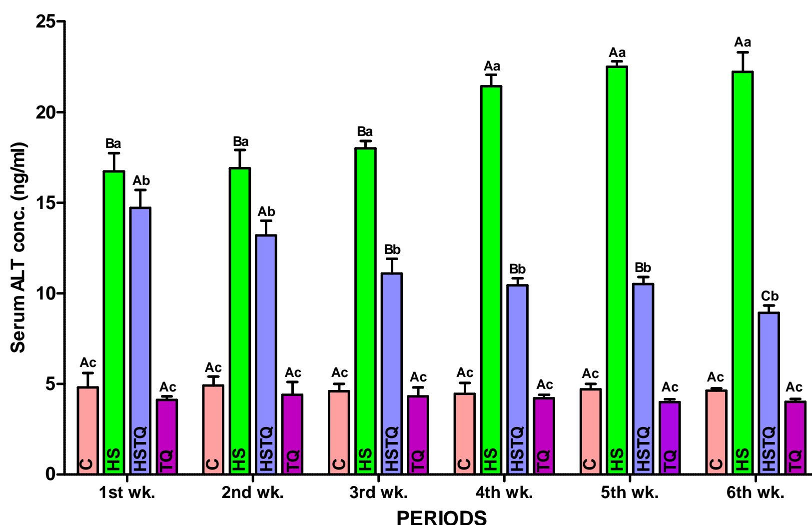

Figure (5): effect of Thymoquinone on serum ALT concentration (ng/ml) in heat stressed male rats.

C: intact male rats orally administered with drinking water daily for 42 days and reared under normal ambient temperature (22-25 ºC). **HS:** heat stressed male rats, orally administered with drinking water daily for 42 days and reared under high ambient temperature(35-40 ºC). **HSTQ:** heat stressed male rats, orally administered with TQ suspension (50 mg/kg, bw) daily for 42 days and reared under high ambient temperature(35-40 ºC). **TQ:** intact male rats orally administered with TQ suspension (50 mg/kg, bw) daily for 42 days and reared under normal ambient temperature (22-25 ºC). Data were presented as Mean ±SD of 10 observations (n=10). Different small letters denote significant difference ($p<0.05$) between groups for each period. Different capital letters denote significant difference ($p<0.05$) between periods for each group.

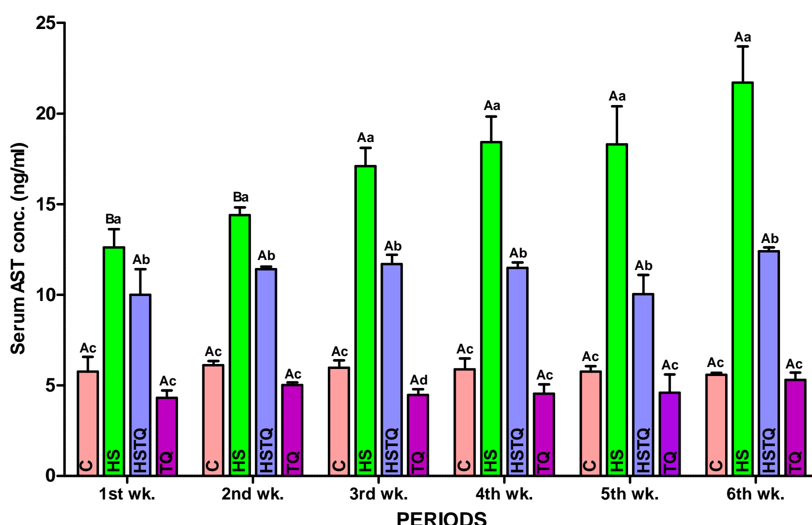

Figure (6): effect of Thymoquinone on serum AST concentration (ng/ml) in heat stressed male rats.

C: intact male rats orally administered with drinking water daily for 42 days and reared under normal ambient temperature (22-25 ºC). **HS:** heat stressed male rats, orally administered with drinking water daily for 42 days and reared under high ambient temperature(35-40 ºC). **HSTQ:** heat stressed male rats, orally administered with TQ suspension (50 mg/kg, bw) daily for 42 days and reared under high ambient temperature(35-40 ºC). **TQ:** intact male rats orally administered with TQ suspension (50 mg/kg, bw) daily for 42 days and reared under normal ambient temperature (22-25 ºC). Data were presented as Mean ±SD of 10 observations (n=10). Different small letters denote significant difference ($p<0.05$) between groups for each period. Different capital letters denote significant difference ($p<0.05$) between periods for each group.

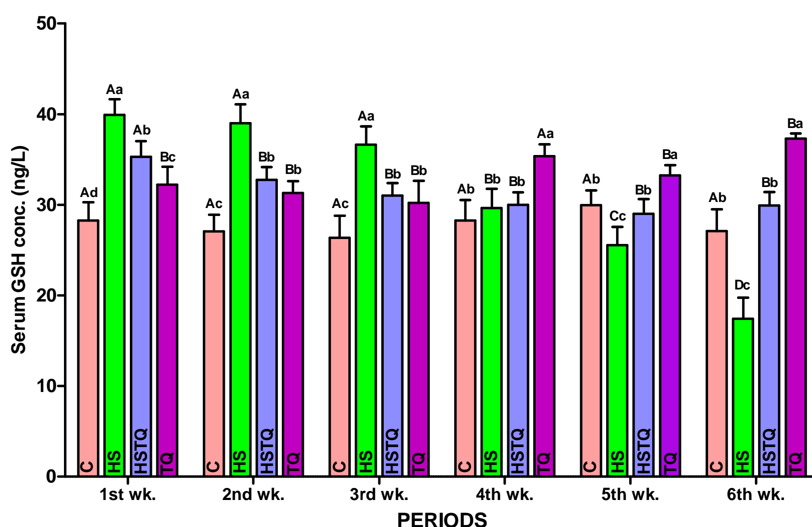

Figure (7): effect of Thymoquinone on serum reduced GSH concentration (ng/L) in heat stressed male rats.

C: intact male rats orally administered with drinking water daily for 42 days and reared under normal ambient temperature (22-25 ºC). **HS:** heat stressed male rats, orally administered with drinking water daily for 42 days and reared under high ambient temperature(35-40 ºC). **HSTQ:** heat stressed male rats, orally administered with TQ suspension (50 mg/kg, bw) daily for 42 days and reared under high ambient temperature(35-40 ºC). **TQ:** intact male rats orally administered with TQ suspension (50 mg/kg, bw) daily for 42 days and reared under normal ambient temperature (22-25 ºC). Data were presented as Mean ±SD of 10 observations (n=10). Different small letters denote significant difference ($p<0.05$) between groups for each period. Different capital letters denote significant difference ($p<0.05$) between periods for each group.

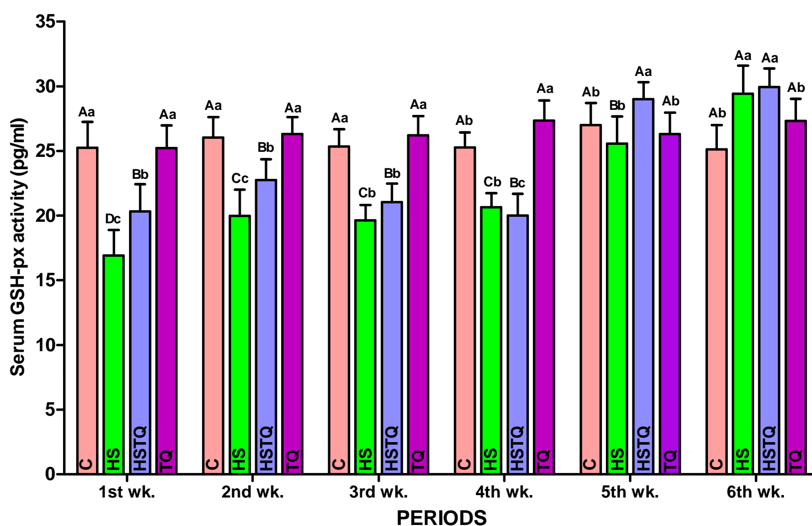

Figure (8): effect of Thymoquinone on serum GSH-px activity (pg/ml) in heat stressed male rats.

C: intact male rats orally administered with drinking water daily for 42 days and reared under normal ambient temperature (22-25 ºC). **HS:** heat stressed male rats, orally administered with drinking water daily for 42 days and reared under high ambient temperature(35-40 ºC). **HSTQ:** heat stressed male rats, orally administered with TQ suspension (50 mg/kg, bw) daily for 42 days and reared under high ambient temperature(35-40 ºC). **TQ:** intact male rats orally administered with TQ suspension (50 mg/kg, bw) daily for 42 days and reared under normal ambient temperature (22-25 ºC). Data were presented as Mean ±SD of 10 observations (n=10). Different small letters denote significant difference ($p<0.05$) between groups for each period. Different capital letters denote significant difference ($p<0.05$) between periods for each group.

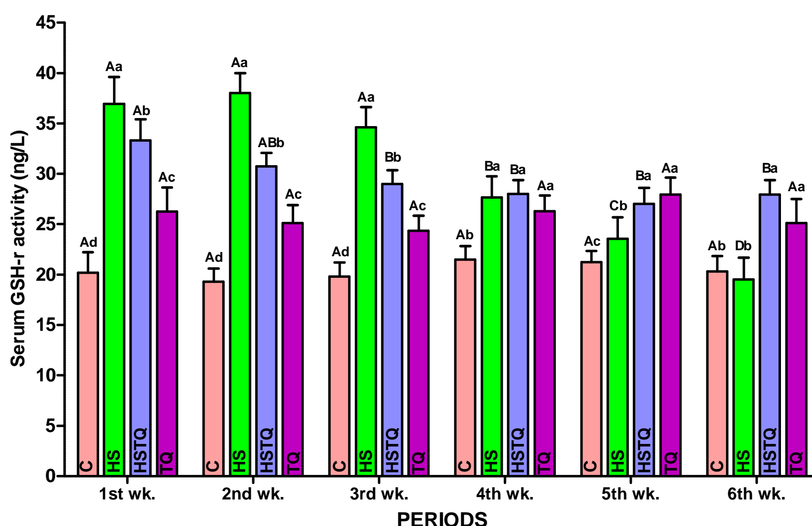

Figure (9): effect of Thymoquinone on serum GSH-r activity (ng/L) in heat stressed male rats.

C: intact male rats orally administered with drinking water daily for 42 days and reared under normal ambient temperature (22-25 ºC). **HS:** heat stressed male rats, orally administered with drinking water daily for 42 days and reared under high ambient temperature(35-40 ºC). **HSTQ:** heat stressed male rats, orally administered with TQ suspension (50 mg/kg, bw) daily for 42 days and reared under high ambient temperature(35-40 ºC). **TQ:** intact male rats orally administered with TQ suspension (50 mg/kg, bw) daily for 42 days and reared under normal ambient temperature (22-25 ºC). Data were presented as Mean ±SD of 10 observations (n=10). Different small letters denote significant difference (p<0.05) between groups for each period. Different capital letters denote significant difference (p<0.05) between periods for each group.

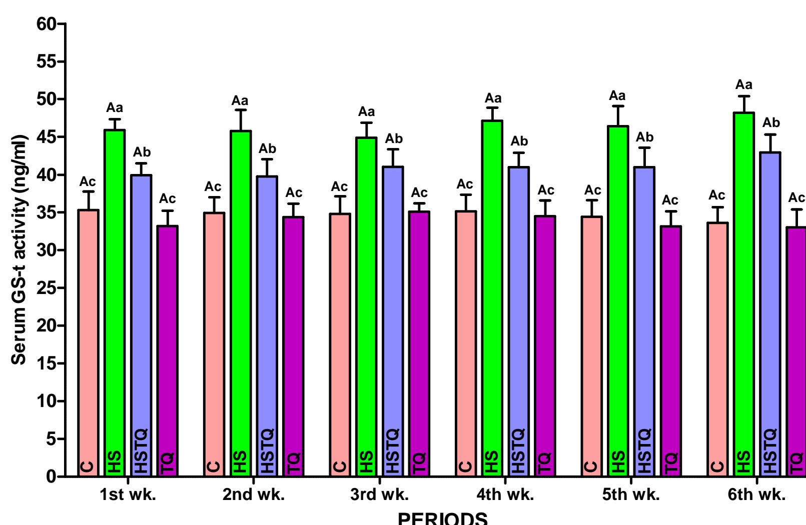

Figure (10): effect of Thymoquinone on serum GS-t activity (ng/ml) in heat stressed male rats.

C: intact male rats orally administered with drinking water daily for 42 days and reared under normal ambient temperature (22-25 ºC). **HS:** heat stressed male rats, orally administered with drinking water daily for 42 days and reared under high ambient temperature(35-40 ºC). **HSTQ:** heat stressed male rats, orally administered with TQ suspension (50 mg/kg, bw) daily for 42 days and reared under high ambient temperature(35-40 ºC). **TQ:** intact male rats orally administered with TQ suspension (50 mg/kg, bw) daily for 42 days and reared under normal ambient temperature (22-25 ºC). Data were presented as Mean ±SD of 10 observations (n=10). Different small letters denote significant difference (p<0.05) between groups for each period. Different capital letters denote significant difference (p<0.05) between periods for each group.