*Hypolipidemic effect of thymoquinone in heat stressed male rats

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Abstract:

The present study has been conducted to investigate the hypolipidemic effect of thymoquinone in heat stressed adult male rats. Seventy two adult male Wistar rats (aged: 80 days, weighted: 162±5.6 g) have been divided into 4 experimental groups (18 animals each), and daily administered for 6 weeks with; drinking water (p.o) under normal ambient temperature (control group); drinking water (p.o) and daily exposed to high ambient temperature (35-40 ºC) for 6 hours (HS group), thymoquinone (50 mg/kg BW, po) and daily exposed to high ambient temperature (35-40 ºC) for 6 hours (HSTQ group), and thymoquinone (50 mg/kg BW, po) under normal ambient temperature (TQ group). At 2, 4, and 6 weeks, six males from each group have been anaesthetized by intraperitoneal injection of 0.3 ml of ketamin and 0.1 ml of xylazin /200 g bw, sacrificed, and blood samples were obtained from abdominal vein for assessment of serum lipid profile (total cholesterol; T-c, triglycerides; TG, high density lipoprotein; HDL-c, low density lipoprotein; LDL-c, and very low density lipoprotein; VLDL-c). During all of the experimental periods, significant increase of T-c, LDL-c, and VLDL-c concentrations have been recorded in HS group compared with control and TQ groups, whereas that of HSTQ group were significantly declined compared with HS group, but still they were significantly higher than that of control and TQ groups. TG and HDL concentration showed significant increase at the same periods. It can be concluded that 50 mg/kg b.w. of TQ suspention drenching for 2, 4, and 6 weeks perform positive role in ameliorating the different complications of oxidative stress produced by heat stress by induction of hypolipidemic effect.

Keywords: Thymoquinone, heat stress, hypolipidemic.

***The research cited from MSc thesis of the second researcher.**

***تأثير الثايموكوينون الخافض للدهون في ذكور الجرذان الناضجة المجهدة حرارياً**

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الخلاصة

أجريت الدراسة الحالية للتحري عن تأثير الثايموكوينون الخافض للدهون في ذكور الجرذان الناضجة والمتعرضة للاجهاد الحراري. تم توزيع 72 جرذا ناضجا بعمر 80 يوما ومعدل وزن 162 ± 5.6 غم عشوائيا على أربع مجموعات متساوية العدد (18 جرذ لكل مجموعة). تمت معاملة الحيوانات لمدة 6 أسابيع على النحو الآتي: جرعت الأولى (C) ماء الشرب بدرجة حرارة الغرفة، وجرعت الثانية (HS) ماء الشرب مع تعرضها الى درجة 35−40 مئوية لمدة 6 ساعات يومياً، وجرعت الثالثة (HSTQ) مستحلب الثايموكوينون (50 ملغم/ كغم من وزن الجسم) مع تعرضها الى درجة 35−40 مئوية لمدة 6 ساعات يومياً، وجرعت الرابعة (TQ) مستحلب الثايموكوينون (50 ملغم/ كغم من وزن الجسم) بدرجة حرارة الغرفة. بعد مرور 2 و 4 و 6 أسابيع من مدة المعاملة، تم تخدير 6 ذكور من كل مجموعة باستخدام الكيتامين والزايلازين (0.3 و 0.1 مل/ كغم من وزن الجسم، على التوالي) وأخذت منها نماذج دم لغرض تقدير حالة الدهون (total cholesterol و TG و HDL-c و LDL-c و VLDL-c). في جميع مراحل الدراسة، أظهرت النتائج ارتفاعاً معنوياً في تراكيز كل من الكولستيرول الكلي و LDL و VLDL في الجرذان المتعرضة للاجهاد الحراري (مجموعة HS) بالمقارنة مع السيطرة والمجموعة المعاملة بالثايموكوينون بدرجة حرارة الغرفة (TQ)، بينما سجلت المجموعة المعاملة بالثايموكوينون تحت ظروف الاجهاد الحراري (HSTQ) انخفاضاً معنوياً بالمقارنة مع مجموعة HS إلا أنها بقيت أعلى من مجموعتي السيطرة و TQ. في حين سجلت مستويات TG و HDL-c ارتفاعاً خلال تلك المراحل. يستنتج من نتائج الدراسة الحالية أن تجريع الثايموكوينون بجرعة 50 ملغم/ كغم من وزن الجسم له دور ايجابي في تعديل تركيز الدهون المتأتي من تعرض ذكور الجرذان للاجهاد الحراري.

الكلمات المفتاحية: الثايموكوينون،الاجهاد الحراري، خافض الدهون.

***البحث مستل من رسالة ماجستير للباحث الثاني**

Introduction

Stress can be defined as an interpreted threat to the individual physiological or psychological integrity, which may results in a physiological and behavioral response. Stress involves both a stressor and response. Stressors is primarily physical and perceived as a threat (physical insult or exertion, noise, heat, cold) or primarily psychological (isolation, interpersonal conflicts, stressful life events) (1).

The imbalance between production of heat inside the body and its liberation could lead to what named heat stress (2). Heat stress is one of the most immportant factors which causes oxidative stress. During oxidative stress, reactive oxygen species (ROS) are constantly produced during metabolism. When reactive oxygen species levels exceeds the threshold value, oxidative stress could be generated and it may causes progressive destruction of poly-unsaturated fatty acids, leading to membrane destruction (3).

There are number of studies showing the effect of severe heat stress on almost every organ in the body (4,5,6). It has been reported that severe heat stress caused changes in activity level, attitude, and behaviour. But, the studies related to the effect of repetitive heat stress of moderate level on structure and biochemical functions of various organs are few (7).

Thymoquinone possess antioxidant effects through enhancing the oxidant scavenger system as well as its potent antiinflammatory mediators prostaglandins and leukotriens (8,9).

Our current opinion is that we should conduct trials with treatment by thymoquinone that may effective against heat stress. The result from this study would provide more insight information on the therapeutic use of thymoquinone in heat stress. Therefore, the present study, aimed to determine the anti-stress effect of thymoquinone in male rats exposed to high ambient temperature. To investigate the hypolipidemic effect of thymoquinone in the heat stressed male rats, the present study focused on the evaluation of serum total cholesterol (T-c), triglycerides (TG), high density lipoprotein cholesterol (HDL-c), low density lipoprotein cholesterol (LDL-c), and very low density lipoprotein cholesterol (VLDL-c) after 2, 4, and 6 weeks of thymoquinone treatment.

Materials and methods

Experimental animals: Mature male Wistar rats have been used in the experiment. Male rats were allowed one week to acclimate to the animal house environment before beginning of experiment. Animals were fed on the standard chow and drinking water *ad libitum* throughout the experiment. Room temperature was maintained at 22±3 °C., the light-dark cycle was on a 12:12 hr with light on at 06:00 a.m. and off at 06:00 p.m. throughout the experimental period.

Experimental Design: Adult male rats have been divided into 4 experimental groups of 18 animals each, and treated for 6 weeks as follow:

Control (intact rats): daily administered with drinking water (p.o) for 6 weeks and reared under normal ambient temperature (22-25 ºC).

HS (heat stressed rats): daily administered with drinking water (p.o) for 6 weeks and daily exposed to high ambient temperature (35-40 ºC) for 6 hrs.

HSTQ (thymoquinone treated heat stressed rats): heat stressed rats daily received thymoquinone (50 mg/kg BW, po) for 6 weeks and daily exposed to high ambient temperature (35-40 ºC) for 6 hrs.

TQ (thymoquinone treated intact rats): intact rats daily received thymoquinone (50 mg/kg BW, po) for 6 weeks and reared under normal ambient temperature (22-25 ºC).

Heat stress has been induced by exposure of male rats to high ambient temperature (35-40 ºC for 6 hrs a day) for 6 weeks. Twenty four hours after the last administration of the tratment, the animals have been processed in the similar manners to those in experiment. Two weeks interval, six male rats from each group have been anaesthetized by intraperitoneal injection of 0.3 ml of ketamin and 0.1 ml of xylazin /200 g bw, sacrificed, and blood samples were obtained from abdominal vein for assessment of serum lipid profile (total cholesterol, TGs, HDL-c, LDL-c, and VLDL-c).

Determination of total serum cholesterol: The procedure was described by (10).

Determination of serum triglycerides (TG): The procedure of evaluation of serum triglycerides was described by (11).

Determination of serum HDL-c conc.: The procedure was described by (12).

Determination of serum VLDL-c conc.: VLDL-c concentration was determined by dividing triglycerides values (in mg /dl) on 5 (13).

Determination of serum LDL-c conc.: when TG concentration not exceeds 400 mg/dl, the following formula is only valid (13):

LDL-c conc. (mg/dL) =total cholesterol – Triglycerides/5 + HDL

Statistical Analysis: All the values are expressed as mean ± SD. Data of the experiment were analyzed using one way analysis of variance (ANOVA 1), using F-test (14). Least significant difference; LSD was carried out to estimate the significance of difference between individual groups. P value less than 0.05 was considered significant.

Results

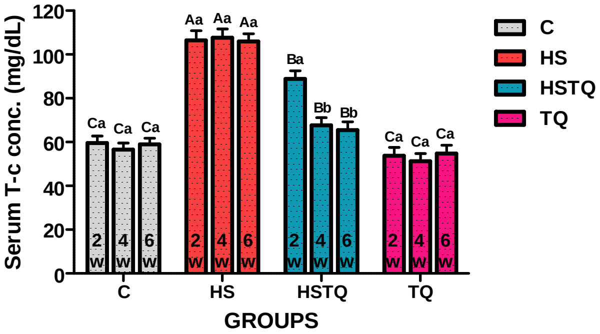
Total cholesterol (T-c): Figure (1-A) illustrate the effect of TQ administration on serum total cholesterol (T-c) concentration (mg/dL) in heat stressed male rats for 2, 4, and 6 weeks. In comparison with control, untreated heat stressed male rats showed significant higher level ($p<0.05$) of T-c concentration at 2, 4, and 6 weeks periods, wheareas TQ treated heat stressed rats showed significant decrease ($p<0.05$) compared with HS group but still significantly higher ($p<0.05$) than control, while TQ treated intact rats registered no significant difference ($p>0.05$) compared with control. In comparison between peroids, HS group showed no significant difference($p>0.05$) between 2, 4, and 6 weeks, whereas HSTQ group was significantly higher ($p<0.05$) at 2 weeks period, and decreased at 4 and 6 weeks periods. On the other hand TQ group recorded no significant difference ($p>0.05$) between periods.

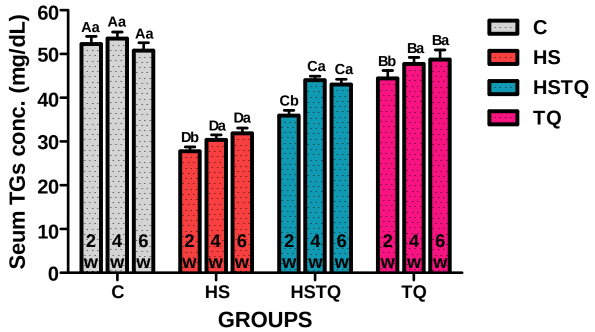
Triglyserides (TGs) concentration: The result illustrates in figure (1-B) shows the effect of TQ administration on serum triglycerides (TGs) concentration (mg/dL) in heat stressed male rats for 2, 4, and 6 weeks. In comparison between groups, control males recorded siginificant ($P<0.05$) higher level among the experimental groups. On the other hand TQ group was siginificantly ($P<0.05$) higher than HSTQ and HS groups, whereas HS group recorded the lowest significant level ($P<0.05$). In comparison between periods (2, 4, and 6 weeks) for each group, 2 weeks period of HS, HSTQ, and TQ groups recorded the lowest levels when compared with 4 and 6 weeks periods which showed no significant difference when compared with each other.

High density lipoprotein-c (HDL-c): Figure (1-C) illustrate the effect of TQ administration on serum HDL-c concentration (mg/dL) in heat stressed male rats for 2, 4, and 6 weeks. In comparison with control, untreated heat stressed male rats (HS group) showed significant lower level ($p<0.05$) of HDL-c concentration at all of the studied periods (2, 4, and 6 weeks). The levels significantly ($p<0.05$) increased in HSTQ group but still lower that that of control, wheareas TQ treated intact male rats (TQ group) showed significant increase ($p<0.05$) compared with other groups. In comparison between peroids, the levels at 4 and 6 weeks periods of HSTQ and TQ groups recorded gradual increase than 2 weeks period in each of the mentioned groups.

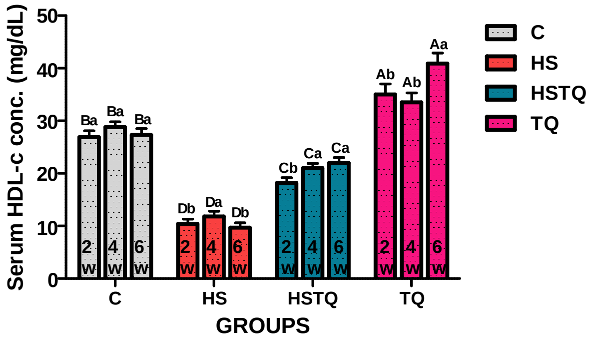
Low density lipoprotein-c (LDL-c): The result illustrates in figure (1-D) shows that LDL-c concentration (mg/dL) significantly ($P<0.05$) increased in heat stressed male rats (HS group) compared with control, whereas heat stressed male rats treated with TQ (HSTQ group) showed gradual decrease during the experimental periods but still significantly ($P<0.05$) higher than control, while intact males treated with TQ recorded significant ($P<0.05$) decrease compared with control. In comparison between periods, control, HS, and TQ groups showed no significant ($P>0.05$) difference between 2, 4, and 6 weeks periods, whereas HSTQ group recorded gradual significant ($P<0.05$) decrease stated at 2 weeks period to the end of experiment.

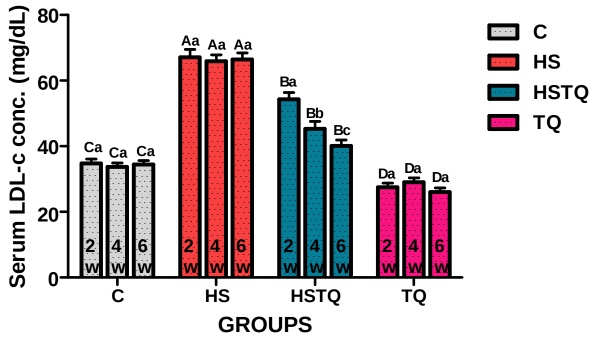
Very low density lipoprotein-c (VLDL-c): The result illustrates in figure (1-E) shows that VLDL-c concentration (mg/dL) significantly ($P<0.05$) increased in heat stressed male rats (HS group) compared with control, whereas heat stressed male rats treated with TQ (HSTQ group) showed no significant ($P>0.05$) difference in comparison with control, while intact male rats treated with TQ recorded significant ($P<0.05$) decrease compared with control. In comparison between periods, the concentration in HS and HSTQ groups significantly ($P<0.05$) increased after 2 weeks of treatment, whereas that of control and TQ groups significantly ($P<0.05$) decreased after 2 weeks of treatment.



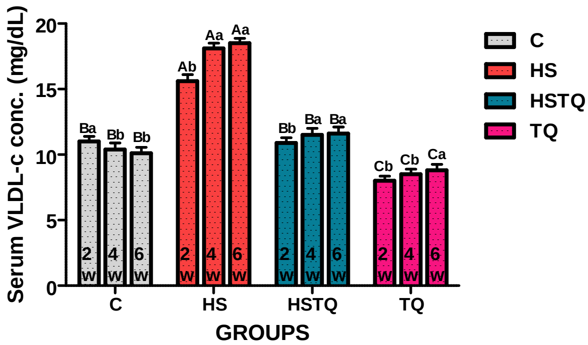


A. Total Cholesterole **B. Triglycerides**





C. HDL-c **D. LDL-c**



E. VLDL-c

Figure (1): Effect of TQ administration on serum T-c (A), TGs (B), HDL-c (C), LDL-c (D), and VLDL-c (E) concentration (mg/dL) in heat stressed male rats on 2, 4, and 6 weeks.
Numbers represent mean ± standard error.
Different capital leters represent significant difference (P<0.05) between groups.
Different small leters represent significant difference (P<0.05) between periods of each group.
C: Intact rats: drenched with drinking water daily for 2, 4, and 6 weeks.
HS: Heat stressed rats: drenched with drinking water daily for 2, 4, and 6 weeks.
HSTQ: Heat stressed rats: drenched with TQ (50 mg/kg, suspended in 500 µl) daily for 2, 4, and 6 weeks.
TQ: Intact rats: drenched with TQ (50 mg/kg, suspended in 500 µl) daily for 2, 4, and 6 weeks.

Discussion

The present study showed that thymoquinone had favorably modified serum lipid profile in male rats with significant decreases in total cholesterol, triglycerides, LDL-cholesterol and VLDL-cholesterol and increased HDL-cholesterol. These results indicate a positive beneficial effect of this compound on lipid and protein metabolism. The lowering of total cholesterol concentration in blood serum may results from the obvious different (inhibitory and stimulatory) effects on many enzymes at the absorption, production and elimination of cholesterol itself or its containing compounds. At the absorption level, the effect of the thymoquinone may be on the Acyl-CoA-cholesterol esterase enzyme that responsible for the absorption of cholesterol in the small intestine (15). Or, the effect of thymoquinone in acceleration of cholesterol elimination by its stimulatory effect on 7-α-hydroxylase which responsible for the conversion of cholesterol into bile acid in the liver (16).

On the other hand, the present results can be attributed to the effect of thymoquinone on cholesterol production through decreasing the activity of β-hydroxy methyl glutaryl-CoA reductase enzyme which responsible for the production of cholesterol in the liver and other body tissues, by converting it into mevalonic acid. This inhibition results in the decrease of the inhibitory effect of cholesterol on protein kinase and the phosphoprotein phosphatase enzymes that responsible for binding of sterol-regulatory element binding protein; SREPS with the DNA (16).

Serum triglycerides lowering effect of thymoquinone could attributed to the decrement of lipid peroxidation, especially lipid hydroperoxidase enzyme, and to the decrement of fatty acids biosynthesis (strearic, olic, and palmitic acids), as well as the elevation of poly unsaturated fatty acids. Ramesh *et al*. (17) found that celery seeds causes decrease in lipid peroxidation in rats. These changes can be explained by the stimulatory effect of the extract on delta-6-desaturase that responsible for the insaturation of fatty acids. This enzyme act to convert the linoleic acid into gamma linoleic. These events result in decreasing the levels of linoleic and arachidonic acids (18). On the other hand, thymoquinone may causes its triglycerides lowering effect by elevating the level of lipoprotein lipase that responsible for the conversion of triglycerides into fatty acids and glycerol (18).

The obvious significant increase of HDL-cholesterol and significant decrease of LDL-cholesterol and VLDL-cholesterol levels compared with the baseline values in our study, refers to the positive efficial role of thymoquinone as hypolipidemic agent. These effects may result from the seed-contained compounds such as monoterpenoids and sequiterpenoids that have hypolipidemic effects (19). As well as its contents of antioxidants such as gamma selenine, 2-methyl propanol, and octa decenamide that responsible for stopping the action of free radicals and prevention the oxidation of linoleic acid (20).

On the other hand, the hypolipidemic effect of thymoquinone may attributed to the formation of new receptors for the LDL and elevation of the catabolic metabolism of these lipids which allows the enterance of cholesterol to the inside of the cells, decrease intestinal absorption of cholesterol, and increase its conversion to bile acid in order to increase its excretion (21). Study of dietary adding *N. Sativa* (30 mg/kg body weight for 20 weeks) showed significant decrease in serum low density lipoprotein cholesterol level, and increase in serum high density lipoprotein cholesterol levels (22). *N. sativa* decreases triglycerides, total cholesterol and increases the healthy cholesterol i.e. HDL cholesterol (23,24,25,26).

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