Effect of harmal seeds on heat stressed chickens

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Abstract: The present study was conducted to clarify the role of harmal seeds *Peganum harmala* in alleviation of heat stress in chickens. The experiment was carried out on 60 chickens that were divided into four equal groups. Group I(gpI) was kept as control under normal conditions (25°C and 50 ± 5 % relative humidity (Rh)), group II(gpII) exposed to daily heat stress period (38°C for 6 hs and 70 ± 5 % Rh), and group III (gpIII) was kept at the same conditions of the control group with adding *Peganum harmala* as 2.5 g/kg ration and group IV(gpIV) exposed to the same conditions of group II with adding *Peganum harmala*(as natural antioxidant) 2.5 g/kg ration. Blood was collected from all groups after one day, one week and 2 weeks. Plasma was separated and stored at – 20°C until used for hormonal and biochemical analysis. Obtained results revealed that plasma levels of corticosterone, glucose and malondialdehyde (MDA) were significantly increased in the heat stressed group. However, a significant decrease in plasma levels of total protein, albumin, uric acid, triiodothyronine (T_3), growth hormone, catalase (CAT) and superoxide dismutase (SOD) were obtained in heat stressed chickens compared with control one. In addition; adding harmal seeds to ration of heat stressed chickens restored normal values of most measured parameters compared with heat stressed group. Decreased lipid peroxidation and enhanced antioxidant activity were also observed in chickens under heat stress and feed ration contained harmal seeds. In conclusion; harmal seeds have good value in minimizing deleterious effects of heat stress.
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1. Introduction

Heat sress is one of the most important stress factors associated with economic losses to the poultry industry in the hot environments. It caused poor growth performance **(Bottje and Harrison, 1985)**, immunosuppression **(Young, 1990)** and high mortality **(Yahav *et al.*, 1995).**

Various physiologic and metabolic changes occurred when chickens are exposed to heat **(Gonzalez - Esquerra and Leeson, 2006)**. To compensate for physiologic disturbances of the body by heat stress, more glucocorticoid is released. Changes in corticosterone levels occurred as a function of environmental stimuli **(korte *et al.*, 2005).** Furthermore, plasma concentrations of T_3 related to environmental temperature **(Yahav *et al.*, 1996)** and levels fall immediately after heat exposure **(Uni *et al.*, 2001)**. The importance of the thyroid gland in adaptation to heat stress is related to the central role that thyroid hormones play in regulation of metabolic rate of birds **(Decuypere and kuhn, 1988).** In addition, the decrease in energy metabolism and feed intake may have effects on the hormone involved in the regulation of growth **(Clemmons and Underwood, 1991).** Heat stress can enhance the formation of reactive oxygen species (ROS), which in turn can cause oxidative injury such as lipid peroxidation and oxidative damage to proteins and DNA **(Mujahid *et al.*, 2007).**

Several studies have been recorded to counteract the deleterious effects of heat stress in chickens by using antioxidants as vitamins C and E **(Sahin *et al.*, 2002),** zinc and vitamin A **(Kucuk *et al.*, 2003)** and taurine **(Shim *et al*,. 2006)**, which act as anti-stress effectors **(Naziroglu *et al.*, 2000)** and prevent certain types of hepatic cellular damage **(Netke *et al.*, 1997).** Although, good results were obtained with these substances, their use might had unfavorable effects in the form of producing problems in tissues of birds. So, it is important to use antioxidant from natural product to control heat stress related problems. Uses of antioxidant from herbal sources (medicinal plants) are very limited because; there is little information about these sources and their active ingredients. Protein isolated from the seeds of *Peganum harmala* alleviated the oxidative stress in the brain, testes and erythrocytes of ccl_4 intoxicated rats. Isolated protein possessed strong antioxidant activity comparable to that of vitamin C **(Soliman and Fahmy, 2011).** In addition, the two major alkaloids harmine and harmala from the seeds of *Peganum harmala* had marked high antioxidant capacity in scavenging or preventive capacity against free radicals induced by oxidation **(Berrougui *et al.*, 2006).** Moreover, intraperitoneal administration of the *Peganum harmala* extract produced significant and dose – dependent hypothermia. In addition; harmine and harmaline, major constituents of the harmala alkaloid,

lowered the body temperature through endogenous 5 – HT stimulation of 5 – H T1 A receptor **(Abdel – Fattah *et al.*, 1995).** Therefore, the present study was carried out to investigate effect of harmal seeds on heat stress in chickens.

2. Materials and Methods

Experimental design:

Sixty chickens 6 weeks old (350 ± 30 gm each) were obtained from private farm at Sharkia Governorate. Chickens were housed in controlled environmental chamber, feed and water were supplied *ad libitum*. After one week of adaptation, chickens were divided into 4 equal groups. The control group (group I) was kept under constant environmental conditions (25°C and 50 ± 5 % Rh), group II exposed to daily heat stress period (38°C for 6 hs and 70 ± 5 % Rh), group III was kept at the same conditions of the control group with adding *Peganum harmala* as 2.5 g / kg ration, and group IV exposed to the same conditions of group II with adding *Peganum harmala* as 2.5g/kg ration **(Abaza *et al.*, 2003).**

Blood sampling :

Blood samples were collected from birds of each group from wing vein (brachial vein) after one day, one week and 2 weeks. Plasma was separated and kept at – 20°c until used for hormonal and biochemical analysis.

Biochemical and hormonal analysis:

Glucose, total protein, albumin, uric acid, MDA, SOD and CAT were determined colourometrically on spectrophotometer according to **Trinder (1969), Peterst (1968), Doumas and Bigg (1972), Artiss and Entwistle (1981), Ohkawa *et al.* (1979), Goldstein and Czapski (1991) and Aebi (1984)** respectively using Bio-diagnostic kits. Corticosterone, T_3 and growth hormone were determined by RIA technique according to **Al – Dujaili *et al.* (1981), Biersack and Hotze (1991) and Raite (1983)** respectively using kits from Monobind, Inc, USA.

Statistical Analysis:

The obtained results were statistically analyzed by one way ANOVA and Least Significant Difference (LSD) at 5 % using Costat Program **(Cohort Software, 2005).**

3. Results

As shown inTables (1,2&3) chickens exposed to heat stress for one day showed significant decrease ($P<0.05$) in the levels of plasma T_3, albumin and SOD.Heat stressed chickens for one and 2 weeks showed significant increase ($P<0.05$) in plasma levels of corticosterone, glucose and MDA and significant decrease ($P<0.05$) of plasma T_3, growth hormone, total protein, albumin, uric acid, CAT and SOD compared to control group.

Data in Tables (2&3) showed that chickens treated with harmal seeds revealed significant increase ($P<0.05$) of plasma SOD after one week and that of plasma total protein and albumin after two weeks compared with control group.

Tables (1,2&3) showed that chickens under heat stress treated with harmal seeds for one day evoked significant increase ($P<0.05$) of plasma SOD.Heat stressed chickens treated with harmal seeds for one week revealed significant increase ($P<0.05$) of blood glucose and significant decrease ($P<0.05$) of plasma growth hormone and albumin compared with control group.Chickens under heat stress treated with harmal seeds for 2 weeks showed significant decrease ($P<0.05$) of plasma albumin, MDA and CAT.

Table (1): Effect of heat stress (38°C for 6hs) and harmal seeds on some hormones of chickens

Parameters / Groups	Corticosterone ng/ml			T_3 ng/ml			Growth hormone ng/ml		
	One day	One week	Two weeks	One day	One week	Two weeks	One day	One week	Two weeks
Control(gpI)	41.1 ± 2.1^{a}	40.4 ±3.6^{b}	41.7 ±3.4^{b}	4.02 ± 0.67^{a}	4.35 ± 0.25^{ab}	4.10 ± 0.31^{ab}	0.43 ± 0.022^{ab}	0.45 ± 0.017^{a}	0.44 ±0.04^{ab}
Chickens under heat stress(gpII)	43.4 ± 3.4^{a}	49.9 ± 2.1^{a}	48.8 ± 2.3^{a}	2.01 ± 0.11^{b}	2.10 ± 0.15^{c}	2.21 ± 0.17^{c}	0.40 ± 0.012^{b}	0.21 ±0.020^{c}	0.23 ±0.010^{c}
Chickens treated with harmal seeds(gpIII)	41.2 ±4.5^{a}	40.06 ± 2.3^{b}	41.3 ± 3.1^{b}	4.17 ±0.21^{a}	4.79 ±0.44^{a}	4.40 ±0.23^{a}	0.45 ±0.021^{a}	0.46 ±0.030^{a}	0.47 ± 0.021^{a}
Chickens under heat stress treated with harmal seeds(gpIV)	40.3 ±2.6^{a}	42.8 ±3.7^{b}	42.9 ±4.6^{ab}	3.76 ±0.35^{a}	3.98 ±0.42^{b}	3.86 ±0.19^{b}	0.41 ±0.011^{b}	0.38 ±0.040^{b}	0.39 ±0.030^{b}

values are expressed as means ± standard errors

^{abc}values in column with different letters are significantly different at $p \leq 0.05$

Table (2): Effect of heat stress (38°C for 6hs) and harmal seeds on antioxidant parameters of chickens

Parameters / Groups	MDA nmol/ml			CAT u/l			SOD u/ml		
	day	week	2 weeks	day	week	2 weeks	day	week	2 weeks
Control(gpI)	1.23 ±0.24^{a}	1.56 ±0.19^{b}	1.45 ±0.21^{b}	1.35 ±0.21^{a}	1.44 ±0.20^{a}	1.52 ±0.14^{a}	0.50 ±0.021^{b}	0.47 ±0.017^{b}	0.53 ±0.051^{a}
Chickens under heat stress(gpII)	1.56 ±0.21^{a}	2.89 ±0.11^{a}	2.37 ±0.13^{a}	1.23 ±0.32^{a}	0.66 ±0.021^{b}	0.73 ±0.04^{c}	0.45 ±0.015^{c}	0.21 ±0.011^{c}	0.28 ±0.021^{b}
Chickens treated with harmal seeds(gpIII)	1.15 ±0.35^{a}	1.43 ±0.20^{b}	1.15 ±0.13^{bc}	1.44 ±0.33^{a}	1.50 ±0.18^{a}	1.56 ±0.13^{a}	0.53 ±0.013^{ab}	0.54 ±0.031^{a}	0.57 ±0.023^{a}
Chickens under heat stress treated with harmal seeds(gpIV)	1.34 ±0.24^{a}	1.37 ±0.13^{b}	0.92 ±0.34^{c}	1.51 ±0.25^{a}	1.34 ±0.31^{a}	1.23 ±0.18^{b}	0.55 ±0.019^{a}	0.46 ±0.031^{b}	0.54 ±0.020^{a}

values are expressed as means ± standard errors

^{abc}values in column with different letters are significantly different at $p \leq 0.05$

Table (3): Effect of heat stress (38°C for 6hs) and harmal seeds on some biochemical parameters of chickens

Parameters / Groups	Glucose mg/dl			Total protein g/dl			Albumin g/dl			Uric acid mg/dl		
	One day	One week	Two weeks	One day	One week	Two weeks	One day	One week	Two weeks	One day	One week	Two weeks
Control(gpI)	173.7 ±6.7^{a}	177.14 ±5.6^{c}	175.9 ±6.8^{b}	4.14 ±0.17^{ab}	4.38 ±0.21^{ab}	4.45 ±0.19^{b}	1.77 ±0.017^{a}	1.96 ±0.042^{ab}	1.89 ±0.024^{b}	5.13 ±0.14^{a}	5.47 ±0.25^{a}	5.42 ±0.31^{a}
Chickens under heat stress(gpII)	168.3 ±7.8^{a}	220.03 ±6.3^{a}	215. 8 ±8.4^{a}	3.89 ±0.23^{b}	2.17 ±0.10^{c}	2.33 ±0.21^{c}	1.65 ±0.023^{b}	0.72 ±0.010^{d}	0.83 ±0.020^{d}	4.83 ±0.25^{a}	3.03 ±0.10^{c}	3.01 ±0.21^{b}
Chickens treated with harmal seeds(gpIII)	175.4 ±6.4^{a}	170.6 ±6.8^{c}	177.6 ±6.9^{b}	4.45 ±0.31^{a}	4.67 ±0.51^{a}	5.87 ±0.32^{a}	1.80 ±0.033^{a}	2.02 ±0.022^{a}	2.03 ±0.05^{a}	5.17 ±0.34^{a}	4.97 ±0.53^{ab}	4.86 ±0.42^{a}
Chickens under heat stress treated with harmal seeds(gpIV)	170.9 ±5.5^{a}	195.2 ±8.9^{b}	185.7 ±7.6^{b}	3.91 ±0.12^{b}	4.02 ±0.23^{b}	4.51 ±0.40^{b}	1.78 ±0.01^{ab}	1.79 ±0.020^{c}	1.77 ±0.040^{c}	4.85 ±0.51^{a}	5.02 ±0.47^{ab}	4.87 ±0.41^{a}

values are expressed as means ± standard errors

^{abcd}values in column with different letters are significantly different at $p \leq 0.05$

4. Discussion.

Heat exposed birds reduced their feed intakes in order to reduce the thermogenic effects associated with nutrient absorption,assimilation and utilization **(McKee *et al.*,1997).**It can negatively affect the defense mechanism in poultry, which can lead to suppressed immune system and increased production of oxygen free radicals **(Bollengier-Lee *et al.*, 1998).** In the present study the heat stressed group showed increased level of blood glucose which is in accordance with those obtained with **Khan *et al.* (2002)** and **Debut *et al.* (2005).** Higher level of blood glucose may be related to heat stress increased glycemia or as a result of increased plasma corticosterone which in turn elicited gluconeogenesis.

Decreased plasma levels of both total protein and albumin observed in the present study under heat stress are in agreement with **khan *et al.* (2002)** who reported a noticeable decrease in the amount of protein in broilers exposed to heat stress.These results may be related to elevation of corticosterone which have elicited gluconeogenesis **(Malheiros *et al.*,2003). Huston and Subha (1969)** reported that high protein level were found at low temperatures than at high temperature.

The obtained data revealed that heat stress decreased plasma uric acid level . These observations are similar to those recorded by **Lin *et al.* (2000).** The decrease in plasma uric acid level might be related to the weight loss of the chickens **(Star *et al.*, 2008),** because there is a positive correlation between plasma uric acid level and body weight loss **(Tsahar *et al.*, 2006).** The reduction in feed intake **(Star *et al.*, 2008)** probably caused the weight loss and related to this the changes in uric acid level.

Chickens under heat stress revealed increased level of plasma corticosterone. Similar results were obtained by **Lin *et al.* (2006)** and **Star *et al.* (2008). Wingfield and Kitaysky (2002)** suggested that corticosterone function as antistress hormone. Increased corticosterone level was to elevate glucose by gluconeogenesis **(Davis *et al.*, 2000)** and suppress glucose uptake of the cells **(Munck *et al.*, 1984).**

The obtained data revealed a significant decrease in the level of plasma T_3 in heat stressed group which is consistent with the result of **Lin *et al.* (2006).** The decreased level of T_3 at high temperature **(Rinaldo and Le- Dividich, 1991)** were consistent with the reduction in heat production in such

conditions **(Quiniou *et al.*, 2001).** On the other hand, **Yalcin *et al.* (2005)** recorded that there were no significant changes in plasma T_3 concentrations of broilers exposed to heat stress.

Exposure of chickens to high temperature reduced plasma level of growth hormone.This result is in agreement with **Collin *et al.*(2002)** who recorded reduction of plasma IGF-1 under high temperature. This decrease may be related to decrease feed intake **(Quiniou *et al.* (2001).**

High temperature increased plasma MDA levels of heat stressed group. Similar results were obtained in serum and tissue of laying hens **(Naziroglu *et al.*, 2000),** Japanese quails **(Sahin *et al.*, 2002)** and broilers **(Pamok *et al.*, 2009).** These observations are in agreement with previous studies suggesting that heat stress induces lipid peroxidation in animal tissues with the production of free radicals **(Bollengier - Lee *et al.*, 1999). Yang *et al.* (2010)** suggested that exposure to high temperature may depress the activity of the mitochondrial respiratory chain, this lead to over production of ROS which ultimately result in lipid peroxidation and oxidative stress. Decreased plasma levels of CAT and SOD in heat stressed chickens are in consistent with the finding of **Altan *et al.* (2003)** who reported increased cellular lipid peroxidation and significant decreases in the activities of antioxidative enzymes CAT, SOD and GPx in broilers exposed to heat stress. Heat exposure act as stress which exaggerated the generation of superoxide anion and increased level of this anion inhibited the activities of the protective enzymes against oxygen radicals, consequently the lipid peroxidation was aggravated **(kokura *et al.*, 2002).** They also added that antioxidant enzymes CAT, SOD, and GSH-Px play a vital role in protecting cellular damage from harmful effects of ROS.

Chickens treated with *Peganum harmala* seeds revealed significant increase of plasma total protien and albumin. These observations is supported by **Abaza *et al.* (2003),** who recorded that harmal seeds as feed additives of broilers improved body weight, weight gain, feed conversion, economical efficiency and increased total protein and globulin.

Chickens under heat stress feed on ration contain harmal seeds showed that plasma levels of most measured parameters approched the control value. Harmal seeds posses beta-Carboline alkaloids which have a significant free radical scavenging capacity **(Berrougui *et al.*, 2006**). In addition; adding harmal seeds to the ration of heat stressed chickens is valuable in alleviating the effects of heat stress, that harmal seeds has antioxidant and antimutagenic properties **(Moura *et al.*, 2007)**.

In conclusion; addition of harmal seeds to the ration of chickens is highly effective to counteract the deleterious effects of heat stress.

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