Antioxidant And Immunostimulant Effects Of *Peganum Harmala* Seeds In Stressed Broiler Chicken

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ABSTRACT

The present study was carried out to determine to any extent *Peganum harmala* seeds extract can antagonise the adverse effect of heat stress on antioxidant and immune status of broiler chicken. The results demonstrated a significant decrease in glutathione content (GSH), total protein, albumin, γ globulin, total leucocytic count and lymphocytes while increased catalase, glutathione-S-transferase (GST) and monocytes were recorded, in birds under heat stress (6 h/day of 39°C and 70% relative humidity). Treatment of heat stressed chicken with 100 mg/kg b.w./day of *P.harmala* seeds extract for 7 days resulted in a significant increase in GSH content, catalase and γ globulin with restoring most values of the resting parameters to the control ones. This study can substantiate the role of *P. harmala* as an antioxidant and immunostimulant for humoral immunity and in improving the status of broiler chicken under heat stress.

INTRODUCTION

Peganum harmala is the most ancient used plant in global flock medicine. The major alkaloids present in the seeds of the *peganum harmala* are beta-carbolines like harmaline and harmine *(1)*. It is well established that *P. harmala* has antibacterial and antifungal effect *(2)*, protective role against lipid peroxidation, potassium cyanide intoxication and whole-body lethal irradiation *(3)*. In addition, it has hypothermic effect mainly through endogenous 5-HT (5-hydroxy traptamine) stimulation of 5-HT1A receptor *(4)*. *P. harmala* was reported to beused in the treatment of haemosporidian infection and neoplasms *(1,5, 6)*. Also, it is used as vasodilatory *(7)* and a disinfecting agent *(8)*.

During summer months, heat stress is of great concern in all types of poultry production and this unfavourable or stressful environmental condition can negatively affect animal's immune system *(9)*, feed consumption, growth rate, hatchability, mortality and the prosperity of the poultry industry *(10)*. It has been shown that heat-induced reactive oxygen species (ROS) formation may be an additional factor that provides molecular changes in DNA, protein, lipid and other biological molecules *(11)*. In addition, it could result in oxidative stress, which in turn lead to cytotoxicity *(12)*, decreased organ function and reduced thermotolerance in old animals *(13)*.

The aim of the present study was to determine the effect of *Peganum harmala* on some anti-oxidant enzymes, total glutathione, serum total protein, albumin and α, β and γ globulins, in addition to the total and differential leucocytic count in broiler chickens exposed to heat stress.

MATERIAL AND METHODS

Experimental design

Eighty Habbard broilers aged three weeks with 550-600 gm average weight were used. The birds were obtained from private farm at Sharkia governorate. Chickens were kept in wire-floored batteries within a thermostatically and humidistatically controlled environmental chamber and supplied with food and water *ad libitum*. After one week of adaptation chickens were divided into four groups of 20 birds each:

The 1st control group

Birds were kept under healthy and constant environmental conditions [27°C and 50% relative humidity (RH)].

The 2nd group

Birds of this group were subjected to a daily cyclic heat stress period (6 h of 39°C and 70% RH) for 7 days.

The 3rd group

This gp. was exposed to periods of heat stress as the previous group with supplying 100 mg/kg b.w./ day of *P. harmala* seeds extract *(4)* in drinking suspension for 7 days.

The fourth group

Birds were kept under healthy environmental condition as control with adding 100 mg/kg.b.w/day of *P. harmala* seeds extract in drinking suspension for 7 days.

Sampling: At the end of experimental period, 10 blood samples were collected from birds in each group by puncture of the brachial vein, 5 blood samples were collected with anticoagulant for blood picture and the other five samples were kept without anticoagulant to obtain clear sera that were kept at –20°C until used for biochemical analysis.

Biochemical analysis

- The total glutathione (GSH) content and catalase activity were was determined colourimeterically *(14, 15)*. While glutathione-S-transferase (GST) activity was estimated spectro-photo-metrically *(16)*. Electro-phoresis for protein fractionation (albumin, α, β and γ globulins) was performed *(17)*.

Leucogram studies

Total and differential leukocytic count were performed using Natt and Herrick diluting solution and Wright stain *(18)*.

Statistical analysis:

The obtained data were analysed using ANOVA computerized programme *(19)*.

RESULTS

The present data showed a significant decrease in total glutathione content and increased both catalase and glutathione-S-transferase activity in broiler chicken under heat stress (Table 1). In groups receiving *P. harmala* the result exhibited a significant elevation in glutathione content and catalase activity while glutathione-S-transferase activity did not differ from the control groups.

Results in Table 2 revealed a significant decrease in total protein, albumin and γ globulin in birds under heat stress, while groups given *P. harmala* showed no significant changes in the concentration of both total protein and albumin whereas a significant increase in of β and γ globulins were noticed in these groups in comparison to the control.

Heat stress resulted in a significant decrease in total leucocytic count and lymphocytes while monocytes increased significantly. At the same time, the use of *P. harmala* resulted in elevation of total leucocyte and lymphocytes with decreasing monocytes to simulate nearly the control group (Table 3).

Table 1. Total glutathione, catalase, glutathione-S-transferase of 21 days old broiler chicken under heat stress treated with *Peganum harmala* extract for 7 days (n= 10).

Parameters / Treatments	Total glutathione µmol/g protein	Catalase µmol/g protein	Glutathione-S-transferase µmol/min./mg protein
Control (C)	29.3 ± 1.6 b	27.0 ± 2.8 b	0.51 ± 0.01 b
Heat stress (H)	20.5 ± 1.9 c	32.1 ± 2.1 a	1.7 ± 0.07 a
Heat stress + *P.harmala* (H + P)	38.7 ± 1.7 a	34.1 ± 2.5 a	0.47 ± 0.02 b
P.harmala (P)	40.1 ± 1.8 a	35.7 ± 1.8 a	0.49 ± 0.01 b

Mean ± S.E

Means in the same column followed by different letters are significantly different (P<0.05).

Table 2. Total protein, albumin, total globulin and its fractionation of 21 days old broiler chicken under heat stress treated with *Paganum harmala* extract for 7 days. (n= 10).

Parameters / Treatments	Total protein (gm/dl)	Albumin (gm/dl)	α globulin (%)	β globulin (%)	γ globulin (%)
Control	4.10 ± 0.31 a	1.50 ± 0.07 a	0.51 ± 0.05 a	0.35 ± 0.02 b	1.70 ± 0.14 c
Heat stress	3.30 ± 0.11 b	0.8 ± 0.07 b	0.55 ± 0.03 a	0.41 ± 0.02 b	1.48 ± 0.07 d
Heat stress + P.harmala	4.80 ± 0.15 a	1.40 ± 0.10 a	0.58 ± 0.04 a	0.54 ± 0.02 a	2.28 ± 0.05 a
P.harmala	4.3 ± 0.26 a	1.35 ± 0.08 a	0.50 ± 0.06 a	0.48 ± 0.03 a	2.00 ± 0.07 b

Mean ± S.E

Means in the same column followed by different letters are significantly different (P<0.05).

Table 3. Total and differential leucocytic count of 21 days old broiler chicken under heat stress treated with *Peganum harmala* extract for 7 days (n= 10).

Parameters / Treatments	Total leucocytic count $10^3/\mu l$	Lymphocyte (%)	Heterophils (%)	Monocytes (%)	Eosinophils (%)	Basophils (%)
Control	26.0 ±1.14 a	29.0±0.70 a	64.0±1.58 a	3.0±0.70 b	2.0±0.31 b	2.0±0.31 a
Heat stress	15.0±0.70 b	21.8±0.80 b	63.2±0.70 a	10.0±0.70 a	3.0±0.44 a	2.0±0.44 a
Heat stress + P.harmala	25.4±0.1.20 a	29.0±0.70 a	65.2±0.86 a	2.0±0.54 b	1.8±0.20 b	2.0±0.31 a
P.harmala	27.0±0.70 a	30.0±0.70 a	65.0±1.14 a	2.0±0.31 b	2.0±0.31 b	1.0±0.31 b

Mean ± S.E

Means in the same column followed by different letters are significantly different (P<0.05).

DISCUSSION

The harmful effects of hyperthermia on organisms have become a matter of concern. ROS resulted from heat exposure are controlled *in vivo* by a wide spectrum of enzymatic and non-enzymatic defense mechanisms. The present data revealed a significant decrease in the level of GSH in heat stressed chickens, which is consistent with the previous results *(20)* which attributed this decrease to the increased oxidation to oxidized glutathione (GSSG), increased degradation or decreased synthesis. Also it was recorded that hyperthermia may reduce body concentrations of antioxidants required for homeostasis *(21)*.

The data present here showed increased activity of both catalase and GST in chicken under heat stress. In agreement with this results, it was found that heat stress caused a significant increase in catalase and GST in rats *(20)* and increased endogenous catalase activity in myocardium after heat stress *(22)*. The elevated activity of catalase may be due to hyperthermia enhanced the cytotoxicity of H_2O_2 in cells and catalase has important role in reduction of H_2O_2 to H_2O and O_2 *(23)*. Elevation of GST activity after heat stress may be derived from the increased lipophilic substances released during oxidative damage to polyunsaturated lipids, such as short-chain aldehydes and alkenals, which are substrates for GST *(24)*.

The obtained data showed increased the GSH content and catalase activity in groups receiving P.harmala. In accordance with this study glutathione, glutathione peroxidase and glutathione reductase were recorded to be significantly higher in melatonin treated rats *(25)*, suggesting that melatonin may effectively normalize the impaired antioxidant status. Where melatonin is active metabolite to 6methoxy-tetrahydro-beta-carboline *(26)*. Furthermore, beta-carboline had potent antioxidants effects and evaluated for cerebral protection against lipid peroxidation *(26)*. Harmaline and harmine was reported to reduce the rate of vitamin E disappearance and exhibited a significant free radical scavenging capacity.

In the heat stressed chickens, the present data exhibited a significant decrease in the concentration of total protein, albumin and γ globulin. Similarly the total protein and albumin were lowered significantly in broiler chickens under toxic stress *(26)*. Heat stress may cause liver injury through increasing the production of ROS levels and oxidative damage to hepatocellular macromolecules

(29) where liver is the main site for plasma protein synthesis.

The present finding revealed a significant elevation of γ globulin in both *P.harmala* treated groups. Similarly γ globulin in rats showed a significant elevation after oral administration of *Rhazya stricta* extract (harmal) and reported no deaths or even toxic symptoms from this extract at dose ranging from 400-4000 mg/kg B.W, indicating the presence of immunopotentiating factor in the plant extract *(30)*.

In heat stressed chicken receiving *P.harmala,* the total protein and albumin concentration returned to their normal values, this may be due to hypothermic effect of *P.harmala* that antagonized the adverse effect of heat stress on these parameters *(4)*.

Total leucocytic count and lymphocytes of chicken under heat stress were lowered significantly in this study. In agreement with this result lymphocytes decreased significantly in the blood of adrenocorticotrophic hormone (ACTH)-injected and heat stressed birds *(9)*. Such leucocytopenia and lymphocytopenia may be attributed to the fact that heat stress increases the activity of the hypothalmo-pituitary adrenal axis in chickens *(31)* causing increase glucocorticoids which induce a mechanism of depressing the lymphopoiesis, arresting lymphocyte formation and producing lysis of these cells, so that in severe stress, the lymphatic tissues are depleted *(32)*. *P.harmala* receiving groups revealed leucocytic picture around the control level, this may be related to melatonin which is metabolized from beta carboline induces metosis and correction of DNA damage *(26)*.

From the obtained results, it was concluded that P. harmala treatment can help to restore the altered antioxidant and immunosuppressive effects on broiler chicken under heat stress.

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الملخص العربى

التأثير المضاد للأكسدة والمحفز للمناعة لبذور نبات الحرمل فى بدارى التسمين المجهدة

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يعتبر الاجهاد الحرارى من أكثر المشاكل الحقلية خطورة. ويرتبط ارتفاع درجة الحرارة أثناء شهور الصيف بزيادة قابلية الطيور للإصابة بالأمراض وزيادة الوفيات.

قد أجريت هذه الدراسة لتحديد إلى أى مدى يمكن لبذور نبات الحرمل أن تضاد التأثير الضار للإجهاد الحرارى على مضادات الأكسدة وعلى الحالة المناعية لبدارى التسمين وقد أثبتت هذه الدراسة نقص مستوى الجلوتاثيون والبروتينات الكلية والزلال والجاما جلوبيولين والعدد الكلى لكرات الدم البيضاء والخلايا الليمفاوية بينما أزداد معدل انزيم الكتاليز والجلوتاثيون اس ترانسفيراز فى الطيور المعرضة للإجهاد الحرارى (٦ ساعات يوميا عند درجة حرارة ٣٩°م و ٧٠% من الرطوبة النسبية).

وعند معالجة الطيور المعرضة للإجهاد الحرارى ببذور نبات الحرمل بنسبة ١٠٠ مجم/كجم من وزن الجسم يوميا لمدة سبع أيام كانت هناك زيادة معنوية فى معدل الجلوتاثيون وانزيم الكتاليز والجاما جلوبيولين مع رجوع معظم القيم الباقية إلى مستوى المجموعة الضابطة.

وهذه الدراسة تعزز دور بذور نبات الحرمل كمضاد للأكسدة وكمحفز للمناعة (المناعة اللاخلوية) وفى تحسين الحالة العامة لبدارى التسمين المعرضة للإجهاد الحرارى.