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SHORT COMMUNICATION

Gambogic Acid Inhibits Hsp90 Expressions in Thiram-Induced Tibial Dyschondroplasia

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ABSTRACT

Tibial Dyschondroplasia (TD) is an important leg problem in fast growing birds that disturbs the proximal tibial growth plate. A study was conducted to evaluate the effects of gambogic acid (GA) on hsp-90 expressions and antioxidant capability in thiram-induced TD chicken. One hundred and fifty day-old commercial broiler chicks were distributed into three groups: Control (A), thiram-induced (B) and GA treated (C). Tibial bone samples were collected at day 7 & 14 to evaluate hsp-90 expressions and liver samples were procured at the end of experiment to determine the antioxidant enzymes. As compared to control, the results demonstrated that significant increased hsp-90 gene expressions ($P<0.05$) in thiram-induced TD chicks on day 7 & 14 contributing to the progression of TD while a decrease in antioxidant capacity of liver on day 14. On administering the GA, the hsp-90 expressions were down-regulated and restored the antioxidant capacity of liver significantly ($P<0.05$). In conclusion, the GA ameliorated the growth plate vascularization in TD-afflicted chicks which provides a new clinical usage of this promising agent against TD.

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INTRODUCTION

Tibial dyschondroplasia (TD) is one of the common skeletal abnormalities in fast growing birds causing major economic losses for avian industry. It is characterized by non-vascularized, un-mineralized and non-viable cartilage in tibial growth plate that fails to form bone (Tian *et al*., 2013; Shahzad *et al*., 2014a). Heat shock protein-90 (hsp-90) is the fundamental component among chaperones playing an essential role in the viability of eukaryotic cells and has been documented to be highly expressed in TD and cancerous cells (Picard, 2002). Recently, the inhibition of hsp-90 activity has been reported in restoring the TD-affected growth plate morphology with the abrogation of lameness depicting its connection with chondrocyte differentiation and growth-plate vascularization (Genin *et al*., 2012).

Gambogic acid (GA) has been used as an anti-inflammatory medicine from thousands of years in China. It is a brownish to orange resin Chinese herb extracted from the *Garcinia hanburyi* tree (genus *Garcinia*, family *Guttiferae*). As one of the major active ingredients and natural compounds, it has been approved for both *in vivo* & *in vitro* clinical trials and exhibits the anti-cancerous properties. It has also been acclaimed for clinical trials by acting as a novel hsp-90 inhibitor (Li *et al.*, 2013).

In present study, the impact of GA, an hsp-90 inhibitor was evaluated in association with hsp-90 and TD in chicken broiler growth plate and its effect on liver antioxidant capabilities was determined.

MATERIALS AND METHODS

The experiment was conducted according to the strategies and approval of the Institution Animal Welfare following the guidelines of Committee of Huazhong Agricultural University Wuhan, China.

One hundred and fifty day-old male broiler chicks (Cobb strain), weighing 52±5 g were purchased from commercial hatchery and maintained under standard hygienic conditions, ambient temperature set at 33ºC and 60% humidity during the experimental period. All the birds were fed normal diet and water *ad libitum*. On day 3, the birds were divided initially into two groups: (A) control group (*n*=50) which received a normal standard diet and (B) thiram group (*n*=100) which was kept on the same diet as to the control group but with the addition of

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40 mg/kg tetramethylthiuram disulphide (thiram) to induce TD. On day 7 post-hatch, 50 birds from thiram group were separated and designated as GA treated group (C). Gambogic acid (>98% purity on HPLC, Batch No. 20140401, Tianjin Shilan Technology Co. Ltd. China) was administered to group C @ 4mg/kg/d intra-peritoneal (IP) from day 7-14. These three groups were raised for 14 days and the number of lame birds in each group was recorded daily. Slaughtering was done on day 07 and 14 and TD lesions were verified and scored as described by Pines *et al.,* (2005). The growth plates from tibiotarsal bones were dissected out, immediately frozen in liquid nitrogen and stored at -70ºC for further analysis.

At the end of experiment, the liver samples from each group were collected and stored at -70ºC for later analysis of glutathione peroxidase (GSH-PX), superoxide dismutase (SOD) and malondialdehyde (MDA) contents. Liver SOD and GSH-Px activity were interpreted in U per milligram of protein (U/mg protein) and liver MDA contents were expressed in nanomoles per gram wet weight of tissue (nmoles/g) by employing commercial reagent kits (Jiancheng Biochem Nanjing, China).

Total RNA was extracted from growth plates of each group and was reverse transcribed into cDNA using a reverse transcription kit (TransGen Biotech Company, Beijing, China). Polymerase chain reaction was performed using the specific primers based on published *Gallus gallus* sequences: for GAPDH forward GCCCAGAACATCATCCCA reverse CGGCAGGTCAG GTCAACA; for hsp-90 forward CTGTGAGGAACTGAT CCCCG reverse TTGCGGTTCTGGGAGTCTTC. The quantification of gene expression was performed by StepOnePlus™ Real-Time PCR System (Applied Biosystems, CA, USA) using SYBER Green RT-PCR kit (Takara Dalian, China) with subsequent thermal cycling parameter: 95ºC for 30 sec, 40 amplification cycles at 95ºC for 8 sec, 59ºC for 30 sec and 72ºC for 30 seconds.

The data was carried out among mean values of control and experimental groups using one way ANOVA followed with student *t* test and presented as mean ±standard error of means (SEM). The differences were considered statistically significant if *P<0.05.

RESULTS AND DISCUSSION

In present study, the expression level of hsp-90 gene and liver oxidative stress was determined before and after GA administration in thiram induced TD. After two days of thiram administration, the birds started showing signs of lameness and pathological characteristics of tibial growth plate at day 7 and 14 were also obvious. As compared to the control group, the TD-affected tibiotarsal bones depicted a non-vascularized and thickened growth plates; however, the birds in GA treated group showed a decreasing pattern in lameness which was more obvious on day-10 post-hatch. A significant (P<0.05) up-regulation in hsp-90 expression was observed in TD-affected birds as compared to control group; however, after gambogic acid treatment, it was down-regulated significantly (P<0.05) (Fig. 1).

The bone-related disorders like tibial dyscho-ndroplasia constitute a major economic loss and pose an animal welfare problem to the poultry industry (Shahzad

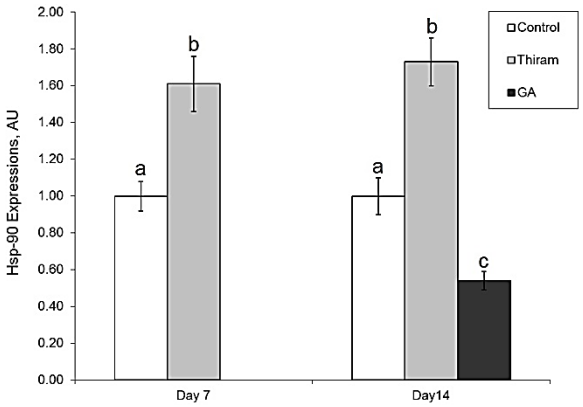

Fig. 1: Effect of gambogic acid administration on hsp-90 gene expressions in thiram-induced tibial dyschondroplasia Real-time quantitative PCR analysis of hsp-90 on day 7 and day 14. Expression levels were normalized to the levels of the geometric mean of GAPDH gene expression. Values (mean±SD; mean of n=4 for each group); results are shown relative to mRNA expression levels from the control group (normal growth plates) set to one corresponding to the n-fold difference., $^{a-c}$P<0.05. AU=arbitrary units.

Table 1: Liver antioxidant activities and MDA contents in thiram-induced (40mg/kg) broiler chicks and gambogic acid (4mg/kg/d) treated group as compared to control (C) on 14 days post-hatch

Parameters	Groups		
	Control	Thiram-induced	Gambogic acid Treated
SOD (U/mg)	133.01±1.3a	88.7±1.6b	124.37±1.2a
GSH-Px (U/mg)	23.54±0.8a	09.23±1.1b	19.03±0.6a
MDA (nmoles/g)	28.35±0.7a	39.8±1.3b	31.7±0.9a

Values (mean±SD) bearing different letters in a row differ significantly (P<0.05). Each group was comprised of 25 birds.

et al., 2015). Thiram, one of the dithiocarbamate is normally used as pesticide and exhibits cytotoxic properties (Sharaf *et al*., 2010; Hussain *et al*., 2011). By interfering with metabolism and development of chondrocytes in fast growing birds, it results into the pathogenesis of TD (Rath *et al.,* 2007). In concomitant with our results, the inhibition of hsp-90 activity has been reported to reduce the hsp-90 levels which resulted into the restoration of vascularization in TD-affected growth plate and ultimately lead to the abrogation of lameness (Genin *et al.,* 2012).

The liver plays a vital role in the metabolism and detoxification of biological substances. In our study, as compared to control birds, a significant decrease (P<0.05) was observed in SOD and GSH-Px, the antioxidant enzymes and an increase (P<0.05) in MDA contents was detected in thiram-induced TD group. These levels were observed in vice versa significantly (P<0.05) in GA-treated group as compared to the thiram group at the end of experiment and the oxidative stress was reduced close to the normal levels significantly (P<0.05). Superoxide dismutase and glutathione peroxidase constitute the major enzymatic oxidative defenses with an important function in protecting the aerobic cells from oxidative stress whereas the lipid peroxidation of cell membrane in case of any stress results in the releases MDA contents as an end product (Perry *et al.,* 2010; Shahzad *et al*., 2014b).

Conclusion: The present study for the first time suggests the hsp-90 inhibition in thiram-induced TD birds with natural hsp-90 inhibitor, gambogic acid. Moreover, the

liver oxidative imbalance caused by thiram can be ameliorated by treating with GA. A further evaluation is required about the prolong use of GA to support the therapeutic use of alternative medicine as hsp-90 inhibitors for the treatment and control of TD.

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Author's contribution: FN and JKL planned the study. FN, MS, KL and JL performed the trial, FN, ZH and DZ analyzed the data. FN wrote the manuscript. All authors read and approved the final manuscript.

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