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Research Article

GELATIN ZYMOGRAPHIC ANALYSIS OF MATRIX METALLOPROTEINASE (MMP) ACTIVITY IN SERUM OF GIRIRAJA FOWL

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ABSTRACT
The present study was conducted to analyze the MMP activity in the serum of Giriraja fowl. Blood was collected from six male and six female healthy Giriraja fowls and serum was separated and stored at -20^{O}C until further use. The serum samples were subjected to Gelatin zymography. A prominent band of 72 kDa (proform), and active form of MMP-2 (62 kDa) were observed. Only one bands observed in both male and female, serum samples. Among these 2 bands, proform of MMP-2 was showing greater gelatinolytic activity. The ratio of proMMP-2/active MMP-2 was found to be 4. None of 3 isoforms of MMP-9 (92,135,220 kDa) was found in the gel in both cock and hen serum samples.

Keywords: *Giriraja Fowl, Serum Gelatin Zymography, MMP*

INTRODUCTION
Matrix metalloproteinases (MMPs) are a large family of calcium-dependent zinc-containing endopeptidases, which are responsible for the tissue remodeling and degradation of the extracellular matrix (ECM), including collagens, elastins, gelatin, matrix glycoproteins and proteoglycan. MMPs are usually minimally expressed in normal physiological conditions and thus homeostasis is maintained. However, MMPs are regulated by hormones, growth factors, and cytokines, and are involved in ovarian functions. Endogenous MMP inhibitors (MMPIs) and tissue inhibitors of MMPs (TIMPs) strictly control these enzymes. Over-expression of MMPs results in an imbalance between the activity of MMPs and TIMPs that can lead to a variety of pathological disorders. Matrix metalloproteinases are excreted by a variety of connective tissue and pro-inflammatory cells including fibroblasts, osteoblasts, endothelial cells, macrophages, neutrophils, and lymphocytes. These enzymes are expressed as zymogens, which are subsequently processed by other proteolytic enzymes (such as serine proteases, furin, plasmin, and others) to generate the active forms. Under normal physiological conditions, the proteolytic activity of the MMPs is controlled at any of the following three known stages: activation of the zymogens, transcription, and inhibition of the active forms by various tissue inhibitors of MMPs (TIMPs). In pathological conditions this equilibrium is shifted toward increased MMP activity leading to tissue degradation. MMPs have now been considered as a promising target for cancer therapy and a large number of synthetic and natural MMP inhibitors (MMPIs) have been identified as cytostatic and anti-angiogenic agents. The present study was carried out to assess the presence of gelatinolytic activity of MMPs in the serum samples of Giriraja fowl through gelatin zymography.

MATERIALS AND METHODS
A total of twelve healthy birds, six males and six females were selected in an organized farm near Orathanadu, Thanjavur. Five milliliters of blood from each bird was collected with the help of a sterilized syringe and needle and collected directly in centrifuge tube. It was centrifuged at 10,000 rpm for 15 minutes at 4°C. The supernatants thus collected were stored at -20 °C till further use. The protein content of the sample was estimated by biuret method with slight modification. A standard curve was built using Bovine Serum Albumin (BSA) as standard. The photometric estimation was carried out with the help of

ELICO SL 207 mini spectrophotometer. Gelatin zymography of serum samples was carried out as per the method of Heussen and Dowdle (1980) with some modifications. And the procedure was as follows. SDS-PAGE was carried out, as described by the method of Laemmli, (1970). The resolving gel (8%) was co-polymerized with 0.3% gelatin solution (final concentration of gelatin in gel was 0.15%) and the electrophoretic run was carried out at 100 V until tracking dye reaches the bottom. Then, renaturation was carried out with renaturation solution (2.5% Triton X -100) for 3 hours on a mechanical shaker with mild agitation. Then, developing was carried out by incubating the gel in developing buffer (10 mM $CaCl_2$, 0.15 M NaCl and 50 mM Tris (pH 7.5)for 18 hours at 37 °C and then stained with 0.25% Coomassie blue for 2 hours, followed by destaining for 1 hour with destaining solution and then, further destaining was carried out with distilled water. Then, calibration of the gelatin zymogram was carried out with human capillary blood gelatinases, as per the procedure suggested by Makowski and Ramsby (1996). A drop of human capillary blood (15-20µL) was obtained by fingerstick puncture and placed in a tarred polypropylene tube. The weight of the blood was determined in an analytical balance and 20 volumes of non-reducing Laemmli buffer was immediately added. The sample was then vortex mixed (30s) and aliquots stored at -20°C.

RESULTS AND DISCUSSION

The gelatin zymography was carried out in serum samples of Giriraja fowl and the results are shown in figure, as:

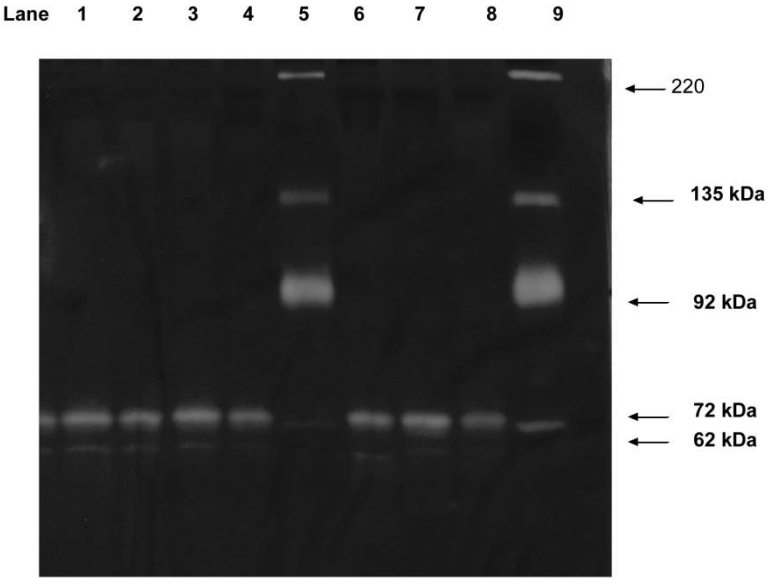

Figure 4.2: Gelatin zymography analysis of serum samples of Giriraja fowl
Lane 1-4: Serum of male birds
Lane 6-8: Serum of female birds
Lane 5 & 9: Human capillary blood markers

A prominent band of 72 kDa (proform), and active form of MMP-2 (62 kDa) were observed. Only one bands observed in both male and female, serum samples. Among these 2 bands, profrom of MMP-2 was showing greater gelatinolytic activity. The ratio of proMMP-2/active MMP-2 was found to be 4. None of 3 isoforms of MMP-9 (92,135,220 kDa) was found in the gel in both cock and hen serum samples, as compared to that of human capillary blood marker MMPs. Ozyigit *et al.*, (2005) reported the presence of 72 kDa MMP-2 in broiler chicken by immunochemistry. Tan *et al.*, (2012) reported that gelatin zymography was used to determine MMP production in condition medium. Under basal condition, a

major band at 72 kDa, and a barely visible band at 62 kDa, corresponding to the prominent and active forms of gelatinase-A (MMP-2) respectively, were detected, where as no other gelatinase activity was evident, including a lack of 92 kDa MMP-2 activity. Packialakshmi *et al.,* (2014) purified bile MMP in chickens by gelatin affinity chromatography and using gelatin zymography, 5 bands were observed (70, 64, 58, 50 and 42 kDa). The bands corresponding to 64, 50, and 42 kDa were identified as MMP-2 using trypsin in -gel digestion and MALT-TOF-MS. Hahn-Dantona *et al.,* (2000) isolated a novel 75-kDa gelatinase from a chicken macrophage cell line, HDII. Biochemical and immunological characterization of the purified enzyme demonstrated that it is distinct from the chicken 72 kDa gelatinase A (MMP-2). A 75 kDa gelatinase is distinct from the only known chicken MMP gelatinase gelatinase A (MMP-2). The apparent electrophoretic mobility of the zymogen from of chicken enzyme (75 kDa) is quite different from that of gelatinase B (92kDa).

In our study it was documented that the only matrix metalloproteinase activity that was found at 72 kDa in the serum samples collected from Giriraja fowl.

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