PE9.10 Exposure of the mouse RAW264.7 macrophage cell line to duck meat extracts: assessment of cytotoxicity and nitric oxide synthesis 71.00

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Abstract - in this study, we used Raw264.7 cells as an experimental model to investigate the effect of duck meat extracts on mouse macrophage function. Raw264.7 cells were treated with various concentrations of poultry extracts with or without endotoxin (lipopolysaccharide, LPS). The results showed that treatments with duck meat extracts alone had no effect on the production of nitric oxide, while the extracts from chicken meat increased nitric oxide released by treated Raw cells. The result was correlated with the increase of iNOS protein expression in the chicken extract-treated cells. However, in the presence of LPS, the chicken extracts did not influence the increase of nitric oxide. Interestingly, duck extract treatment reduced LPS-induced nitric oxide production, which was associated with the decrease of iNOS expression. Base on these findings, it is highly likely that duck meat has more effects on the inflammatory pathway than chicken meat under the condition of infection. Our study provides primary scientific evidences regarding the relationship of some immune responses and duck meat from the traditional Chinese medicine point of view.

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Index Terms - cytotoxicity, duck, extract, immune, nitric oxide.

I. INTRODUCTION

DUCK is one of the major poultry consumed in Taiwan. In addition to as a good source of variety of nutrients, duck meat provides high levels of antioxidants as well [1]. Some studies also showed that the extracts from duck meat inhibited the activity of angiotensin converting enzyme (ACE), which may have some preventing effect on hypertension [2]. But, from the point of view of traditional Chinese medicine, duck meat is “toxic” that may enhance inflammatory response, and might cause some harmful effects to the patients with wounds. However, there is no such scientific data supporting this thought. Nitric oxide (NO), which is a key biological messenger and inflammatory mediator, plays an important role in a variety of physiological and pathophysiological processes, such as macrophage-mediated cytotoxicity, blood vessel dilatation, smooth muscle relaxation and neurotransmission [3-4]. The objective of this study was to assess the cytotoxicity and nitric oxide synthesis of the mouse RAW264.7 macrophage cell line which was exposed to duck meat extracts.

II. MATERIALS AND METHODS

A. Sample preparation

Meat sample with deionized water (1:3 w/w) was cooked in a pressured cooker for 1 h. After deboning and homogenization, homogenized sample was extracted with ethyl acetate (EA). Layers of water extracts and ethyl acetate (EA) extracts were separated. EA extracts were then concentrated with n-hexane to extract lipids. In addition, homogenized samples were digested with pepsin stimulatingly (pH 2.0, 37ºC for 20 h) or lipase (pH 8.1, 37ºC for 60 min) and frozen dried. Samples which were resolved in buffers and filtered were then added into cell culture systems.

B. Cell culturing

Mouse macrophage cell line RAW264.7 (ATCC TIB-7) which was obtained from the ATCC/ NHRI Cell Bank (Taiwan) (BCRC 60001) was cultured in 90% Dulbecco`s Modified Eagle`s Medium (DMEM) which containing 4.5 g/L glucose, 4 mM L-glutamine, 1.5 g/L sodium bicarbonate, 10% fetal bovine serum, 100 unit/ml penicillin and 100 £gg/ml streptomycin. Culture passage was conducted twice every week. During passage culture, cells were washed using

phosphate buffer saline (PBS) for 3 times and added with a trypsin-EDTA solution. After setting at 37ºC for 3 min, cells were released, mixed with new medium, transferred to a new bottle, and finally centrifuged for 5 min (3000 rpm, swing rotor). After suspending in a new medium, cells which were adjusted at 2.5¡Ñ105 cell/ml were then seeded into 96-well microplates (200 £gl/well; 5¡Ñ104 cell/well). After setting at 37ºC for at least 12 h, poultry meat extracts were added into cell culture medium in the absence or presence of lipopolysaccharide (LPS) and conducted the further assay.

C. Cytotoxicity assay

Cytotoxicity of poultry meat extracts on macrophages was determined using MTT assay. Cells were incubated in 96-well microplates (200 £gl/well) with MTT (5 mg/ml) for various periods of time. The nitrite concentration in the cell culture medium serves as a surrogate measure of NO synthesis and macrophage activation. After adding new media (100 £gl/well) and 10 £gl MTT (5 mg/ml), cells were incubated at 37ºC for 1 h. Livable cells would transform tetrazolium into formazan (purple color). After adding 100 £gl 0.04 N HCl isopropanol to dissolve, the absorbance of the solutions was read on a spectrophotometer at 570 nm while using 630 nm as a reference.

D. Nitric oxide (NO) assay on RAW264.7 macrophages

According to the method of Taira et al. (2009), after culturing, the nitrite-levels, as an NO indicator in the medium, which were produced by Raw264.7 cell was determined by the Griess Reagent System (Promega Co., Madison, WI, USA). Briefly, aliquots of 100 £gl of medium were in mixed in a 96-well microplate and then 50 £gl of a 1% sulfanilamide solution containing 5% phosphoric acid, were added. The reaction mixture was incubated for 10 min at room temperature and then 50 £gl of a 0.1% N-(1-naphyl) ethylenediamine dihydrochloride solution were added. After 10 min of incubation, the absorbance at 540 nm was measured. NaNO2 was used as the standard to calculate the nitrite concentration.

III. RESULTS AND DISCUSSION

A. Cytotoxicity assay of duck meat extract

In this study, water extracts and ethyl acetate (EA) extracts of duck meat which were diluted with cell culture medium at different dilution factors were added with cells and incubated for 24 and 48 h, and cell viabilities were then determined using MTT assay.

Figure 1. Cytotoxicity of duck meat ethyl acetate extracts on mouse RAW264.7 macrophages

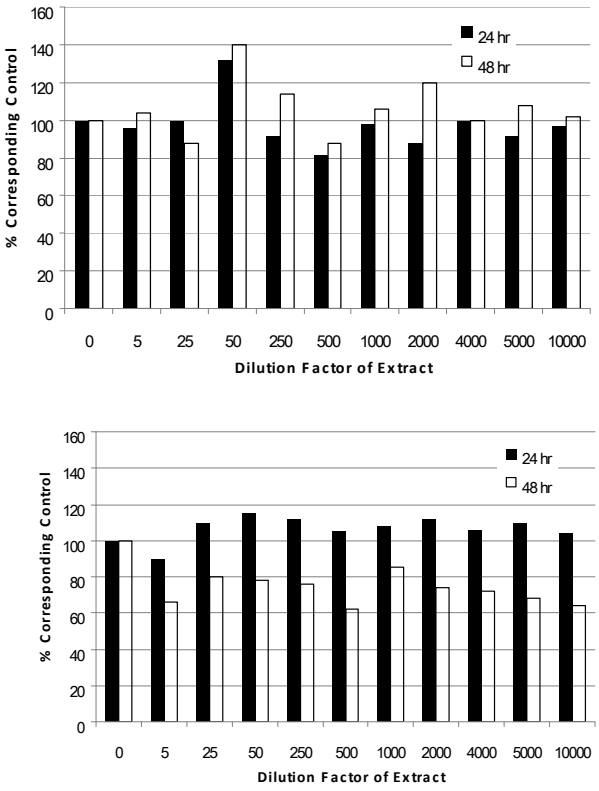

Figure 2. Cytotoxicity of duck meat water extracts on mouse RAW264.7 macrophages

As showed in Figures 1 and 2, cell viabilities were not influenced by the EA-extracts of duck meat at various dilution factors of 5 to 10000. However, cell viabilities of those which were cultured with duck meat water extracts for 48 h were lower than the samples without addition of extracts. This result implies that there might be some materials containing in this water extracts of duck meat and then influenced cell viability.

B. Determination of activation of duck meat extract on macrophages

Macrophages might be activated to express inducible nitric oxide synthase (iNOS) and produce excessive amounts of nitric oxide (NO). Lipopolysaccharide (LPS) which was produced by Gram positive bacteria would activate macrophages [5]. In this study, it was used to evaluate whether poultry (i.e. duck or chicken) meat extracts would activate macrophages. RAW264.7 cell was first incubated in media which containing LPS and poultry meat extracts and the nitrite concentrations in media were determined. The result showed that cell cultures which were incubated with duck meat extracts

(either water-extract or EA-extract) for 24 or 48 h did not influence the nitrite concentrations in media.

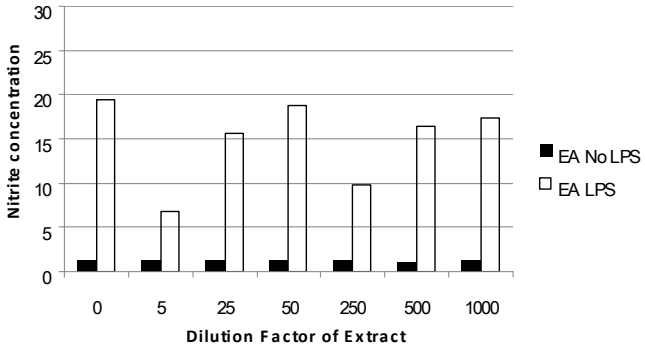

Figure 3. Influences of duck meat ethyl acetate extracts on nitric oxide released by mouse RAW264.7 macrophages

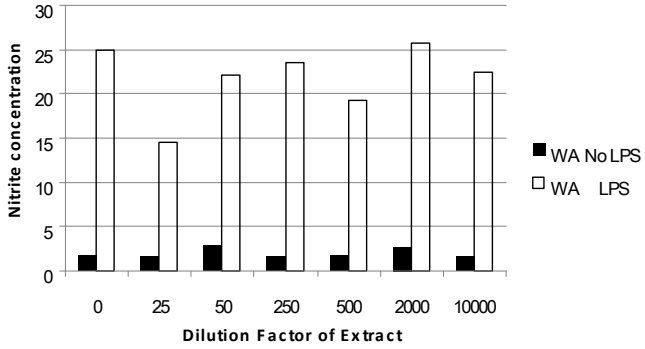

Figure 4. Influences of duck meat water extracts on nitric oxide released by mouse RAW264.7 macrophages

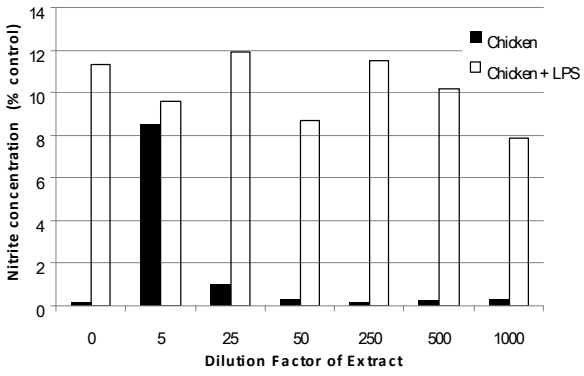

Figure 5. Influences of chicken meat extracts on nitric oxide released by mouse RAW264.7 macrophages

However, higher levels of duck meat extracts lowered the production of nitrate in the presence of LPS (Figures 3 and 4).

C. Comparison of activation of duck or chicken meat extracts on macrophages In order to evaluate whether there might exist some different activation on macrophages, extracts from duck or chicken meat were both evaluated in this study. The result showed that chicken meat extract at higher concentration (i.e. dilution factor=5¡Ñ) significantly increased the production of NO by macrophages in the absence of LPS (Figure 5). However, addition of LPS and chicken meat extracts simultaneously did not influence macrophage significantly. Duck meat extracts or chicken meat extracts did not influence protein expression of Raw cycloxygenase.

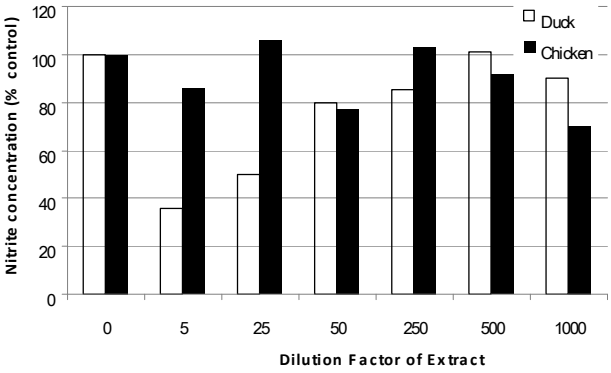

Figure 6. Comparison of duck and chicken meat extracts on nitric oxide released by LPS-induced mouse RAW264.7 macrophages

IV. CONCLUSION

In conclusion, at higher concentrations, duck and chicken meat extracts would influence the macrophage functions. However, duck meat extracts might reduce the production of NO by macrophages in the presence of bacterial endotoxin lipopolysaccharide (LPS). Further studies are needed to access the relationship and mechanism between duck meat consumption and inducible inflammation.

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