

Abstract

Siamese crocodile (*Crocodylus siamensis*) blood has been added value and increase its usage. However, the basic research knowledge about chemical compositions of the crocodile blood and its compositions has been limited. The present study was the second year in total of three years studying. The Siamese crocodile serum proteome was studied. Albumin and immunoglobulin were depleted and separated with 2-Dimensional electrophoresis (2DE) combined with Mass spectrometry that using for marker proteins or peptides in crocodile serum. The data demonstrated no difference of protein profile between the fresh serum and freeze-dried serum. All of large protein molecules were not eliminated from the serum. There were 90 protein spots resolved by 2DE ranging from 20 – 200 kDa with isoelectric point (pI) of 4 to 10. On the basis of the pI, 30 protein spots were categorized in acidic, forty protein spots in neutral and 15 protein spots in the basic region of 2DE gel image. Of all these spots, 16 brightly stained protein spots were determined by MALDI-TOF/MS analysis. These findings suggest the data of Siamese crocodile blood about the serum proteome. The donation of crocodile blood was studied by development of blood collection process on animal life maintains in captive Siamese crocodile. The total blood volume of 30 crocodiles at average weight 22.71 kg was averaged 598.33 millilitres per crocodile. The blood collection from captive breeding crocodiles which maintain life were study from 20 crocodiles which average weight was 27 Kilograms, 191 centimeters in length and 4-5 years of age. All crocodiles were divided in 4 groups which 5 for each group. Control group were collected 10 milliliters every weeks for 12 weeks. Experimental group 1: withdrawn 150 ml of blood on week 1 and 12 and collected blood 10 mL every week starting from week 2-11. Experiments group 2: withdrawn 150 ml of blood at week 1, 12 and collected blood 10 mL week 4 and 8. Experiments group 3: withdrawn 150 ml of blood at week 1 and 12. The result showed that experimental Group 1-3 after blood collection 150 ml 1 weeks hematological value is not significant difference from control group that collect 10 mL ($p > 0.05$) in all the time of the experiment. Biochemical value at first and last week of experiment showed

VI

that 150 ml or about 25 percent of total blood volume has no effects on crocodile health in each time of collection. Study the cytotoxic activity against cancer cell lines (breast cancer cell (MCF-7) lung cancer cell (COR-L23) liver cancer cell (HepG2) and cervical cancer cell (Hela) by SRB assay. HIV protease and HIV 1 Integrase were also tested. Crocodile blood in the whole blood, serum and plasma (PE) dissolved with PBS, DMSO, 95% ethanol, 10%HCl and 10%NaHCO₃ had no cytotoxic activity against four cancer cell lines with IC₅₀ values of > 100 µg/ml. While crocodile blood dissolved in 10%HCl more exhibited than cytotoxicity against cancer on crocodile blood dissolved in solvent another. The crocodile blood dissolved in 10%HCl exhibited breast and liver cancer cell line (MCF-7, HepG2) with an IC₅₀ value 60 – 90 µg/ml. The HIV-1 protease and integrase (HIV-1 IN) activities had no HIV-1 protease and HIV-1 integrase (IC₅₀ > 100 µg/ml). The extract of crocodile blood no displayed antioxidant activity by DPPH assay with EC₅₀ >100 µg/ml, and the anti-inflammatory activity by nitric oxide inhibition assay had no activity too. However, the plasma of crocodile blood in DMSO showed IC₅₀ value = 12.91 µg/ml and the whole blood, serum and plasma (PE) of in 10%HCl with IC₅₀ 30-50 µg/ml against anti-allergy activity. These results of overall activities showed that the extract of crocodile blood good exhibited anti-allergy activity. Furthermore, the crocodile blood dissolved with 10%HCl showed increased values of activities. Thus the extraction of crocodile blood should be in acid condition. To study of the effect of crocodile serum on neurite outgrowth and the amount of MEK-1 in PC12 culture. Serum obtained from 10 crocodiles (5 males and 5 females) were freezed dried and experimented. PC12 was cultured for cytotoxic effect of the dried serum. The percentage of cell bearing neurite outgrowth were counted in totally of 100 cells. The amount of MEK-1, produced while cell proliferated and differentiated, was evaluated by the enzyme immunoassay method. The concentrated crocodile serum was directly toxic to neurone cell culture at the IC₅₀ of 25.26 mg/ml. The diluted serum at the concentration of 4 mg/ml potentiated proliferation and neurite outgrowth with the percentage of 9.04 ± 3.42 more than the negative control group with ngf 2 ng/ml (3.05± 1.32%) but lesser than the positive control group with ngf 50 ng/ml (14.81 ± 3.79%) (p <0.05). The amount of MEK-1 were also increase in the treatment of 4

VII

mg/ml group as $204 \pm 28.91\%$ and the positive control group as $253.26 \pm 34.2\%$ of negative control group ($p < 0.05$). The diluted serum of 2 and 0.4 mg/ml had no effect on neurite outgrowth and MEK-1 synthesis. To study on the production process of low fat sausage fortified with dietary fiber from herb and inulin and with angiotensin converting enzyme inhibitor derived from hydrolysate of crocodile blood. Types and quantities of spices were investigated. The formula with higher amount of finger root and lower amount of turmeric powder and kaffir lime peel was found to have the highest acceptance. Fat and inulin quantities used in the selected formula were investigated. 25% fat reduction (25F), 25% fat reduction with 3% inulin (3I), 25% fat reduction with 6% inulin (6I), 25% fat reduction with 9% inulin (9I) and full fat (control) sausage formula were evaluated for cooking loss, emulsion stability, color, texture, hardness, springiness, cohesiveness and sensory test. The sausage of 6I was shown the highest consumer preference ($p \leq 0.05$). The substitution of fat with crocodile blood was studied. The results indicated that the sausage with 15% fat reduction was found to received highest score from consumers. Proximate analysis revealed that this formula had moisture, fat, protein fiber, carbohydrate and ash values of 65, 3.5, 16.5, 0.72 and 7.5% by weight, respectively. The sausage was packed in LDPE bag, sealed under vacuum and kept at 4°C . The results indicated that the sausage was accepted from consumers throughout storage time and can be kept for at least 7 days.

Key word: Crocodile, Crocodile blood, Medicinal animal, Chemical compound, Protein, Carbohydrate, Lipid, Antimicrobial peptide, Anti-cancer, Anti-HIV, Cancer, AIDS, Anti-free radical, Free radical, Immunity, Wound healing, Anti-malignant, ACE-inhibitor, Brain effect, Crocodile product, Functional food