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CATEGORY: TRANDITIONAL VETERINARY MEDICINE
TOPIC:

Development of novel earthworm powder and investigation of its therapeutic effects in animals

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Background and Objectives:
Earthworms are well-known soil decomposers. Because they are polyphagous animals and their cast contains a large amount of nutrients, application of earthworms in composting has been studied extensively. Earthworms have been also studied for their therapeutic effects against human diseases. In particular, dried earthworm powder, called "Earth Dragon" or "Jiryu" (in Japanese),has been used as an antipyretic and diuretic treatment since ancient times. Furthermore, earthworms have fibrinolytic enzymes, and dried earthworm powder has been found to have health benefits for humans. However, the powder manufactured by conventional methods does not have high fibrinolytic activity because of the heat treatment involved in the final step. Thus, we have developed a novel production system that involves pressure treatment. Furthermore, we characterized the powder and investigated its therapeutic effects after oral administration in animals.

Materials and Methods: The earthworms, *Eiseniafetida* Waki, and its lyophilized powder were kindly provided by Waki Pharmaceutical Co., Ltd., Nara, Japan. The conditions for the high-pressure treatments were as follows:100 MPa at 60 C for 16 h by using SHP100-50A (Shinada Co.,LTD.,Niigata, Japan). Lumbrokinase (LR) activity (same as fibrinolytic activity)was measured at 37 Cby measuring the formation of *p*-nitroaniline (*p*NA) at 405 nm with a Corona Absorbance Microplate Reader MTP-310Lab (Corona Electric Co., Ltd., Ibaraki, Japan). S-2251 (Sekisui Medical Co., Ltd., Tokyo, Japan) and S-2288(Sekisui Medical) were used as substrates for plasmin and tissue-plasminogen activator (t-PA), respectively. A fibrin plate assay was performed as follows: Hundred milligrams of fibrinogen was mixed with 10 mL of FP buffer (50 mM Tris-HCl, 93 mM NaCl, 1.66 mM $CaCl_2 \cdot 2H_2O$, 0.96 mM $MgCl_2 \cdot 6H_2O$). The mixed solutionwas centrifuged and the supernatant was transferred into a plastic plate. Then, 50 μL of thrombin (20 NIH units/ml) was added into the plate. Therapeutic effects in

animals were investigated by determin ingreactive oxygen metabolites-derived compounds(d-ROMs) and biological antioxidant potential (BAP)test.