

## Phytoestrogens (Genistein and Diadzein): Distribution in Tempeh and Their Endocrine-Modulating Effects in Mice

R Jegathambigai<sup>1</sup> and O. Iekhsan<sup>2</sup>

<sup>1</sup>Department of Biotechnology, Faculty of Applied Sciences, AIMST university, Sungai Petani, Kedah, Malaysia

<sup>2</sup>Department of Molecular Medicine, University of Malaya, Kuala Lumpur, Malaysia

Regular phytoestrogen consumption results in an increase in the circulating concentrations of endogenous estrogens. Hence the evaluation of the potential benefits and risks offered by soy phytoestrogens requires investigation of their potency and sites of action, when consumed at natural dietary concentrations. The study determined the distribution of the phytoestrogens, genistein and diadzein (free and total) in tempeh, and also their endocrine-modulating effects in mice. The concentrations of genistein and diadzein (free and total) were determined by silica-gel column fractionation, HPLC determination and by acid hydrolysis and ethanol extraction of dried tempeh respectively. The endocrine-modulating effects of genistein and diadzein were determined by the oral administration of the standard compounds at low (1 or 5 mg/kg) and high (10 mg/kg) dose regimens, continuously for 14 days for adult males and subcutaneously for 7 days for neonatal females. Morphological changes in testes, epididymis and thyroid of adult males, and ovaries of neonatal females were determined. Hormonal parameters such as serum and testicular testosterone and serum total triiodothyronine (T<sub>3</sub>) were determined in males. The concentrations of genistein and diadzein present in the extracts of the tempeh was correlated to the equivalent amount of standards used in the *in-vivo* study. The amount of free genistein and diadzein in tempeh was found to be 10.95 mg and 5.37 mg/100g wet weight of tempeh. The amount of total genistein and diadzein was found to be 32.4 mg/100g and 23.8 mg/100g wet weight of tempeh. *In-vivo* study on the endocrine-modulating effects of genistein and diadzein showed degenerative changes such as presence of multi-nucleated giant cells, immature germ cells and reduction in seminiferous tubule diameters in the testes and reduction in the number of sperms in most tubular lumens of epididymis in both groups. There was no significant morphological changes in the thyroids of the both treated groups. The ovaries of the females exhibited poly-oocyte follicles with increased incidence in the high-dose groups. The serum and testicular testosterone levels and total T<sub>3</sub> were also significantly reduced in

both dose groups with greater reduction in the high-dose groups. The incidence of degenerative changes was found to be higher in the high dose groups of both males and females. The amount of tempeh extracts which could produce the high dose effects in mice was found to be 25 mg/kg and 50 mg/kg for genistein and diadzein standards respectively. Human phytoestrogen levels should be evaluated and the consequences of the adult and early developmental life in females should be examined. Hence exposure of phytoestrogens causes significant morphological and hormonal changes in mice. Such exposures may affect the developing fetus and the male reproductive system of mice.