ABSTRACT

SUSSI ASTUTI. The Effects of Isoflavone-riched Soybean Flour, Zinc (Zn) and Vitamin E on Fertility of Male Rats as Animal Model. Under direction of DEDDY MUCHTADI as chairman and MADE ASTAWAN, BAMBANG PURWANTARA, and TUTIK WRESDIYATI as members.

The objectives of this research were to (1) evaluate the effects of isoflavone-riched soybean flour, zinc (Zn) and vitamin E on fertility of male rats, (2) evaluate the effects of isoflavone-riched soybean flour with different levels of isoflavone on fertility of male rats.

Prior the in vivo experiment, isoflavone-riched soybean flour was defatted using n-hexane. Quantitative analysis of isoflavone on the defatted-soybean flour was conducted by HPLC, while antioxidant activity on the defatted-soybean flour was conducted by DPPH method. Total isoflavone on the defatted-soybean flour (TKI-RL) was considered as the basic concentration that used to calculate isoflavone dosages for the in vivo experiment.

Male and female weaning Sprague Dawley rats (21 days old) were used as the animal model in the experiment. On the 1st stage of the experiment, the male rats were given basic diets containing isonitrogen and isocalories with 15% of dietary protein from casein. Thirty male Sprague Dawley weaning rats (21 days old) were divided into six groups and treated with isoflavone-riched soybean flour, Zn and vitamin E in different combination. Isoflavone-riched soybean flour (3mg/day) was given by oral administration, whereas Zn and vitamin E were mixed with the basic diet. The treatment was conducted for 2 month. On the other hand, thirty female Sprague Dawley rats were only given the basic diets containing 15% of dietary protein from casein to observe the fertility of male rats. After 2 months, both male and female rats were mixed (1:1). Vagina swabs on the female rats were taken every morning to evaluate the conception rate and to detect spermatozoa by Giemsa dyes. Once the spermatozoa detected on the female rats (the 1st day of pregnancy, D1), the male rats were sacrificed by dislocation of cervical bones (dislocasio cervicalis), then all the parameters were tested. On the day 15th of pregnancy, the female rats were sacrificed (D15), the conception rates and total fetus were counted.

On the 2nd stage of the experiment, male rats were given basic diets containing isonitrogen and isocalories with 10% dietary protein from casein. Twenty five male of Sprague Dawley rats were divided into five groups and treated with isoflavone-riched soybean flour by oral administration with different levels (dosage). The treatment was conducted for 2 months. On the other hand, twenty five female Sprague Dawley rats were only given the basic diets containing 10% of dietary protein from casein for 2 months to observe the fertility of male rats. After 2 months, both male and female rats were mixed (1:1). Vagina swabs on the female rats were taken every morning to evaluate the conception rate and to detect spermatozoa by Giemsa dyes. Once the spermatozoa detected on the female rats (the 1st day of pregnancy, D1), the male rats were sacrificed by dislocation of cervical bones (dislocasio cervicalis), then all the parameters were tested. On the day 15th of pregnancy, the female rats were sacrificed (D15), the conception rates and total fetus were counted.

The data were tested by analysis of variance (ANOVA) using Completely Randomized Design (RAL) to evaluate the effects of the treatments on the tested-parameters. To observe the difference between experiments, the data were then continued for Duncan Multiple Range Test (DMRT).
Quantitative analysis of isoflavone compound in TKI-RL by HPLC indicated that total concentration of isoflavone (daidzein, genistein, and glisitein) was 2.35 g/100g dried-matter, and the IC$_{50}$ was 51.96 μg/ml. The result of the 1st stage of the experiment revealed the synergisms effects between isoflavone-riched soybean flour with Zn and vitamin E on the male rats. The effects observed were the reduction of testical MDA level, recovery of testical SOD activities, stability the content of Cu,Zn-superoxide dismutase (Cu,Zn-SOD) in spermatocytes and early spermatids cells of seminiferous tubules by immunohistochemical technique, increasing of sperm concentration and rate of motility, increasing of serum testosterone and total spermatogenic cells in the seminiferous tubules of testes. In general, complete treatment of isoflavone-riched soybean flour, Zn and vitamin E on male rats resulted in better fertility in comparison with single treatment and the other combination. However, combination the three components did not affect significantly on relative weight of testes, as well as to the conception rate and the number of fetus on female rats. The best fertility of male rats was on the group given isoflavone 3 mg/day, Zn 6.14 mg/kg diet and vitamin E 100 mg/kg diet.

The results of the 2nd stage of the experiment showed that the treatment of TKI-RL on the male rats given isoflavone with dosage of 0 mg/day, 1.5 mg/day, 3 mg/day, 4.5 mg/day, and 6 mg/day did not affect significantly on spermatozoa abnormality. Treatment of isoflavone 6 mg/day resulted in infertility (copulation rate and conception rate on female rats were 100% and 0%, respectively). The higher dosage of isoflavone caused the increasing of concentration of the serum testosterone and Leydig cells of the male rats. The best treatment resulted in the best fertility was on the group given isoflavone 1.5 mg/day indicated by the increasing of relative weight of testes, rate of motility, sperm concentration and total spermatogenic cells in the seminiferous tubules of testes, inhibiting the formation of lipid peroxide which was indicated by decreasing the testical MDA level, recovery of testical SOD activities, and stability the content of Cu,Zn-SOD in the spermatocyte and early spermatid cells as indicated by immunohistochemical technique.

**Key words**: isoflavone-riched soybean flour, Zn, vitamin E, fertility, male rats