THE IMMUNOGLOBULIN (IgG) ANALYSIS OF IMPLANTATION MICE (*Mus musculus* L.) POST OVARIECTOMY AFTER TREATMENT OF BLACK SOYBEAN FLOUR EXTRACT (*Glycine soja*)

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Abstract

Black soybean is one type of plant that has estrogenic activity, it can act as a source of natural exogenous estrogen. Decreased estrogen hormone in the body due to ovariectomy and menopause, it effects on immune response (IgG). The purpose of this study was to determine the effect of black soybean extract on immune response (IgG) of implantation mice post ovariectomy. Twenty-eight mice were grouped into 4 groups, group 1 (negative control), group 2 (positive control with the administration estradiol concentration 50 ppm), group 3 and 4 were treatment group with the administration of black soybean flour extract doses of 0.31 g/ml and 0.63 g/ml. The data were analyzed using One Way Anova and Duncan Multiple Range Test (DMRT) (p<0,05). The treatment with administration of black soybean flour extract for 10 days in implantation mice post ovariectomy showed that a dose of 0.31 g/ml and dose of 0.63 g/ml had an effect on increasing significantly the immune response (IgG), i.e 0,12987and 0,12996 respectively.

Keywords: *immunoglobulin (IgG), ovariectomy, Mus musculus, black soybean (Glycine soja)*

1. INTRODUCTION

Black soybean is a type of Leguminosae plants it contains isoflavone compounds. Isoflavones are active compounds of phytoestrogens that have estrogenic activity, they can bind to estrogen receptors in the body and replace the function of endogenous estrogen hormones. These compounds play a role as a source of exogenous estrogen in the body (Tanu, 2005; Badziad, 2003).

Decreased estrogen in the body can be caused by menopause and ovariectomy. Ovariectomy is the process of removing the ovaries in the female reproductive system (Alagwu and Nneli, 2005). The alteration of hormonal and immune system can affect to implantation development. Estrogen can modulate the immune system during implantation (Ortona *et al*., 2015). Some immune factors that influence implantation are chemokines, cytokines, TGF-β, IL-6, IL-11, EGF, B cells, T cells and antibodies. One type of antibody that influences implantation is immunoglobulin G (IgG). IgG plays a role in the implantation process, which can activate NK cells that regulate the implantation stage (Xie et al., 2005). IgG plays a role in migrating trophoblast cells that invade the endometrial and protect the embryo from foreign substances in body (Bernard *et al*., 1997; Chin *et al*., 2015). Thus, decreasing estrogen hormone will cause proinflammation which is characterized by low IgG levels that affect to asymptomatic autoimmunity (Singh *et al*., 2011; Engdahl *et al*., 2018).

Deficiency estrogen can be increased by phytoestrogens. The administration of soy phytoestrogen compounds with a concentration of 26% can increase IgG and IgM levels in ovariectomized rats (Shalaby and Elgawish, 2015). The administration genistein treatment of 250 ppm concentration in rats was able to significantly increase NK cell activity, B cell counts, and T cells (Guo *et al*., 2002). Based on the background, the purpose of the study to carry out the effect of administration of black soybean flour extract (*Glycine soja*) on immune response (IgG) of implantation mice (*Mus musculus* L.) post ovariectomy.

2. RESEARCH METHOD

Research site and setting

This research conducted from November 2018 to April 2019 in the Zoology and Biotechnology Laboratory, Faculty of Mathematics and Natural Sciences, University of Jember.

Black Soybean Flour Extract

The production of black soybean flour extract begins was roasted at a temperature of 40-45ºC for 2-3 days. Then grinder and macerated for 2x24 hours and put into the rotary evaporator for ± 4-5 hours with a temperature of 80ºC. The next step was placed in a porcelain cup and placed on a water bath with temperature 70ºC for ± 8 hours to get paste of black soybean flour extract.

Experimental animals

The animal's model were 28 mice (*Mus musculus* L.) age 2 months and weight 200 gram. It collected from Surabaya Veterrian Farma (Pusvetma). The mice were divided into 4 groups of 7 mices per group. The mices were acclimatized at room temperature (25-30 ºC) with standard feed and water *ad libitum*.

Unilateral Ovariectomy (ULO) Preparation

Unilateral ovariectomy (ULO) is removing one of ovaries in mice. The mice are anesthetized and made incision to open the muscular layer of the abdominal area slowly. To close of the muscular layer by stitching, and disinfection by using povidone iodine in the incision area. The last stage was injected 0.05 ml of Levofloxacin antibiotics. The treatment of wound can be done by administering paracetamol for 1 week ad libitum.

Experimental procedure

The mice were randomized 4 groups: group 1(negative controls); group 2 (positive controls by treatment extradiol); group 3 and 4 were treatment with the administration of black soybean flour doses of 0.31 g/ml and 0.63 g/ml respectively. The administration of estradiol and black soybean flour extract which were given orally (gavage method) using a sonde. The treatment started on the 41st day post ovariectomy to 51st day. Then, the mating was carried out on the 51st day at night and is counted as the 0th pregnancy day.

Blood Serum Preparation and Immune Response Analysis (IgG) using ELISA Method

Blood sampling was carried out on days 0, 41, 51 and 59 from orbital veins of mice. The IgG analysis was carried out by using elisa analysis. Antigen coating by 50 µl black soybean flour extract incubated at 37ºC for 12 hours. The solution was removed and washed with PBST 3 times @ 250 µl. The Next step added 50 µl primary antibodies in a ratio of 1: 100 and incubated at 37ºC for 2 hours. Add secondary antibodies 50 ul in a ratio of 1: 2500 and incubated for 37° C for 1 hour. The solution is discarded and washed by using 250 µl PBST. TMB (Tetramethylbezidine) substrate was added in a dark room and incubated at room temperature. The absorbance measurement (OD) was carried out by using ELISA reader with wavelength of 450 nm.

Statistical analysis

Statistical analysis was performed using One Way ANOVA SPSS version 23.0 and Duncan Multiple Range Test (DMRT). Differences were considered to be significant at $p<0,05$.

3.RESULTS AND DISCUSSION

Immune response (IgG) was analyzed using ELISA method. The results of population IgG can be seen in Figure 1. The results of IgG in Figure 1. Showed both the treatment group given estradiol and black soybean flour extract can increase the immune response (IgG) significantly.

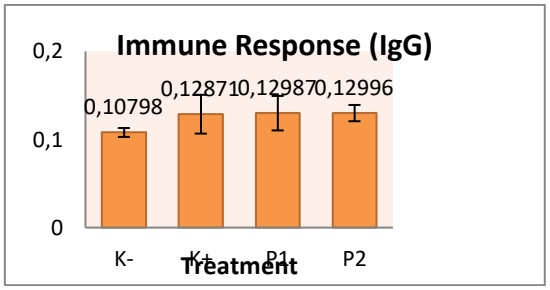


Figure 1. The average immune response (IgG) of implantation mice post treatment of black soybean flour extract. K-: without treatment, K +: Estradiol concentration of 50 ppm, P1: Dose 0.31 g/ml, P2: Dose 0.63 g/ml.

Based on analysis of the One Way Anova test, the treatment of black soybean flour extract affected average immune response (IgG) significantly between treatment groups. The analysis of DMRT test in Figure 1. shows that there is a significant difference on average immune response (IgG) in negative control with the treatment group. The average immune response (IgG) in treatment group was higher (K +: 0.12871^{b} ± 0.02203; P1: 0.12987^{b} ± 0.01955; P2: 0.12996^{b} ± 0.00924) compared to the negative control (0 , 10798^{a} ± 0.00504).

Figure 1. shows that the assumption are estradiol and black soybean flour extract have same role as exogenous estrogens so they can IgG in treatment animal that have estrogen deficiency. Estradiol has almost the same affinity with isoflavone compounds and both are immunomodulators in the body's immune response. Estradiol and phytoestrogens are able to bind estrogen receptors both ERα and ERβ found in all body tissues, have estrogenic activity and act as Asselective Estrogen Receptor Modulators (SERMs) (Beshay and Carr, 2013). In addition, estradiol has a high affinity and is controlled by the presence of estrogen receptor bonds that can be found in all body tissues including specific and non-specific immune cells. Estradiol compounds can increase IgG concentrations due to the binding of estradiol with membrane receptors in the target tissue so it can affect humoral and cellular immune responses in body or can be immunomodulators of the body's immune system. Immunomodulator is a certain natural or synthetic compound it can stimulate and modulate the immune response in body. Phytoestrogen compounds can increase CD4+ and IgG in estrogen deficiency conditions by stimulating the response of immunocompetent B cells through the signaling pathways of estrogen receptor binding (Shalaby and Elgawish, 2015; Sumalatha *et al*., 2012). This is also showed by following graph of average immune response (IgG) (Figure 2).

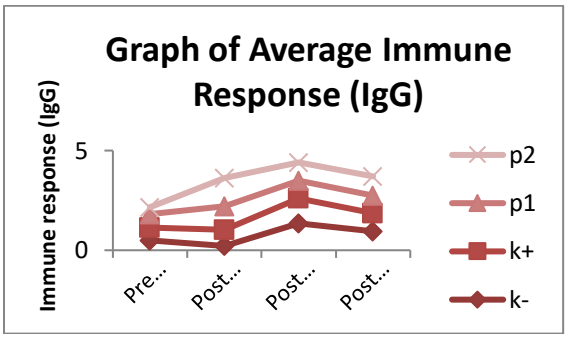


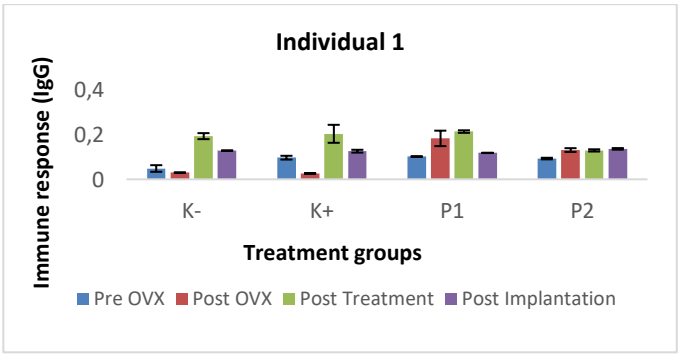
Figure 2. The Graph of average immune response (IgG) of implantation mice post treatment of black soybean flour extract. K-: without treatment, K +: Estradiol concentration of 50 ppm, P1: Dose 0.31 g/ml, P2: Dose 0.63 g/ml.

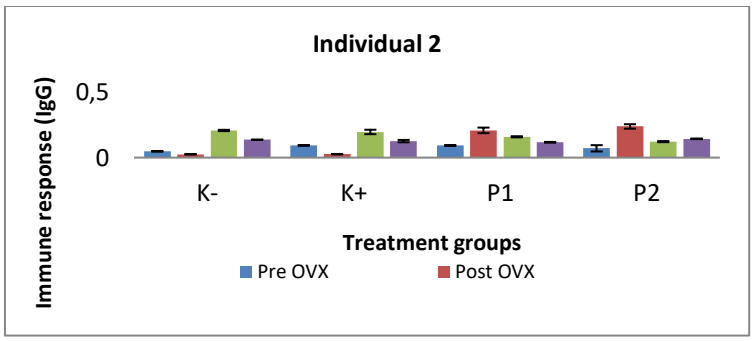
The results of the average of immune response (IgG) graph (Figure 2) show that the immune response in the group treated with both estradiol and black soybean flour extract had an average value of IgG which its increase. The results of the average immune response (IgG) were also strengthened by the results of individual immune responses (Figure 3) which the seven individuals in the treatment group (positive controls, P1 and P2) showed higher IgG values compared to the control group overall negative. Based on Figure 3, some individuals post ovariectomy showed an increase in the immune response (IgG) while there were some individuals after treatment by estradiol and black soybean flour extract administration are showing the immune response (IgG) have increase. So, the administration of exogenous estrogen intake can act as a substitute for endogenous estrogen.

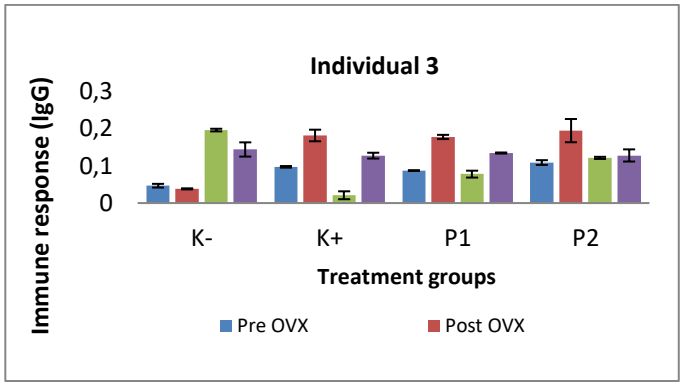
Figure 2. shows that the treatment animals after being treated and implantation

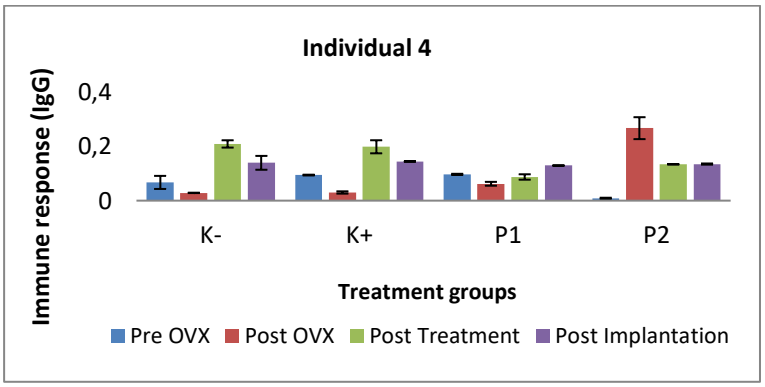
showed the average of the immune response (IgG) have decreased. The influence of hormonal changes can affect immune system in body on development of implantation. Treatment animal are more likely to experience hormonal imbalances due to the influence of the estrous cycle. The estrous cycle is a period when animals are ready to mate. The process of estrus until implantation occurs is very closely related to hormonal changes. Sex hormone levels are very fluctuating due to variations in the concentrations of LH and FSH during the reproduction process. Thus, it affects the roles of sex hormones in regulating implantation. One of the sex hormones that play a role in implantation is estrogen. estrogen hormone will modulate immune system during implantation by regulating the development of T cells and B cells. The influence of hormonal fluctuations is likely to affect the decreased activity of immune cells in the body when regulating implantation (Bouman *et al*., 2005). When estrogen deficiency conditions cause proinflammation characterized by low IgG levels, in addition, it affects the inhibited activity of immune cells including decreased activity of macrophage cells, NK cells, mast cells, IFNγ secretion cells, inhibition of differentiation of B cells and regulation of chemokines and cytokines as well as IL-4 secretion. IL-4 is one of the Th2 cytokines it modulates differentiation and proliferation of B cells in producing antibodies (Cutolo *et al*., 2004; Grimaldi *et al*., 2002).

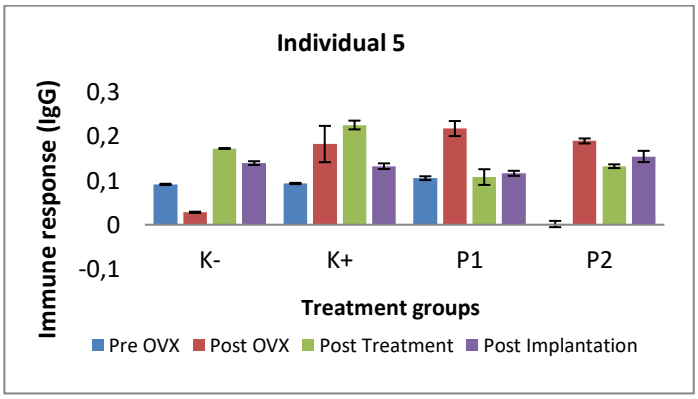
In addition, both have a structure and affinity similar to endogenous estrogen. The following graph of immune response of individual implantation mice post administration black soybean flour extract can be seen in Figure 3.

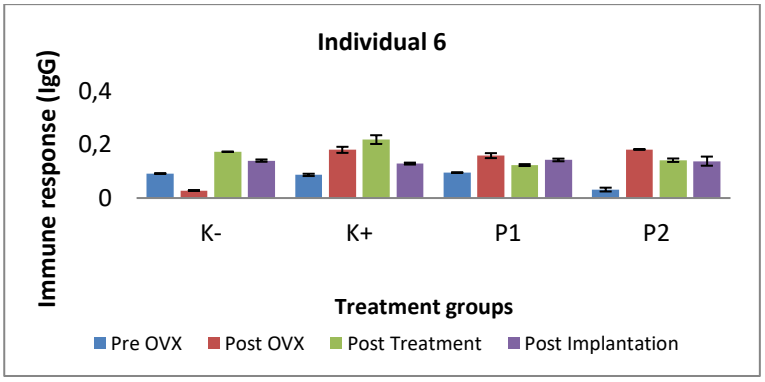





Individual 3
Immune response (IgG)
0,3
0,2
0,1
0
K-
K+
P1
P2
Treatment groups
Pre OVX
Post OVX


Individual 4
Immune response (IgG)
0,4
0,2
0
K-
K+
P1
P2
Treatment groups
Pre OVX
Post OVX
Post Treatment
Post Implantation


Individual 5
Immune response (IgG)
0,3
0,2
0,1
0
-0,1
K-
K+
P1
P2
Treatment groups
Pre OVX
Post OVX
Post Treatment
Post Implantation


Individual 6
Immune response (IgG)
0,4
0,2
0
K-
K+
P1
P2
Treatment groups
Pre OVX
Post OVX
Post Treatment
Post Implantation

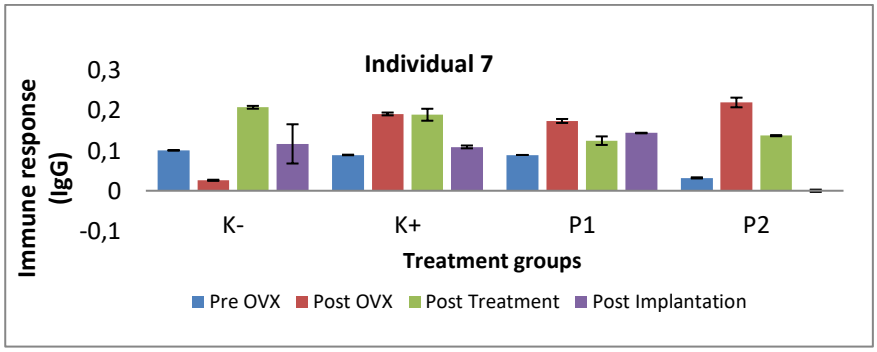


Figure 3. Immune response (IgG) of individual implantation mice post treatment of black soybean flour extract. K-: without treatment, K +: Estradiol concentration of 50 ppm, P1: Dose 0.31 g/ml, P2: Dose 0.63 g/ml.

Figure 3. shows the administration of estradiol and black soybean flour extracts, it can act as exogenous estrogens will increase the activity of immune cells, it's antibody (IgG) of treatment animals. So, this can affect the transmission of antibodies when implantation occurs. The role of IgG on implantation will help to migrate trophoblast cells in invading endometrial and as a protection against foreign substances in the body. Some individuals post implantation also show increased IgG. However, there are individuals who show decreased immune response (IgG). The decreasing and increasing on IgG is due to hormonal differences in each individual. Each individual is given the same treatment, with the same type of strain and weight. So, in this case is the main factor in regulation of immune system in the body is estrogen. Hormonal influence during reproductive process takes place especially on implantation, it affects the differentiation and activation of immune cells in the body.

4. CONCLUSION

The treatment of black soybean flour extract for 10 days in implantation mice post ovariectomy showed that a dose of 0.31 g/ml and dose of 0.63 g/ml had an effect on increasing significantly the average of immune response (IgG) i.e 0,12987; 0,12996 respectively.

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