The Body Weight and Thickness in Chicken Embryo Incubated for 10 Days Induced by Turmeric Rhizome Simplisia

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Abstract: The zygote develops into an embryo after going through the process of organogenesis. During organogenesis, there is also the process of angiogenesis, the formation of blood vessels. However, the process of angiogenesis can be hampered by several factors including the presence of foreign compounds that enter the parent body. One compound that is believed to have an angiogenesis inhibiting effect is curcumin from the turmeric rhizome simplisia. Inhibition of angiogenesis due to curcumin induction can have an impact on embryonic organogenesis in terms of embryonic body weigth and thicness so this study was conducted to determine the teratogenic effects of curcumin of turmeric rhizome simplisia on chicken embryos. This study used fifteen unincubated chicken eggs divided into three groups, the negative control group, the group injected with turmeric rhizome simplisia at a dose of 0.48 mg/gr and DMSO 2% as much as 0.25 mL, and the group injected with turmeric rhizome simplisia at a dose of 0.96 mg/gr and DMSO 2% as much as 0.25 mL. All groups were incubated for 10 days at 38 °C. The parameters measured were embryo body weight use digital scales and measurement of the body thicness with calipers. The results showed that the turmeric rhizome simplisia at dose of 0,48,mg/gr cause died in one of incubated chicken egg and at dose of 0.96 mg/gr cause two of five incubated chicken egg was died. The left eggs from group of dose 0.48 mg/gr and 0.98 mg/gr have decreased of body weight and thicness compared with negative control group. It was showed that turmeric rhizome simplicial in this doses have effect in decrease body weight and thickness of chicken embryos.

1 INTRODUCTION

Animals have the ability to multiply through a reproductive process. Reproduction is the process of breeding the living creatures, starting from the fertilization that happen between egg-cell with the sperm cell so that it forms a new individual called zygote. Zigot evolved into embryos after the process of Organogenesis and was born in the form of fetus (Subronto and Tjahayati, 2001). In the process of organogenesis, there is also an angiogenesis (process of blood vessel formation). This process involves Vascular Endothelial Growth Factor (VEGF) which is a group of growth factor with broad function as vascular permeability used as angiogenesis markers during organogenesis process, one of which when forming Heart. However organogenesis process can be hampered by several factors including the presence of foreign compounds that enter the parent body. One of the compounds believed to have an organogenesis inhibitory effect is curcumin derived from the Simplisia rhizome of turmeric. Curcumin is the largest part of the yellow pigment contained in the turmeric rhizome which has several biological benefits such as antioxidant, anti-inflammatory, and Antineoplastic (Nurrochmad, 2004).

According to Maniago Research (2014) which uses Chorio Allantois Membrane (CAM) Duck eggs age 10 days, it was known that turmeric can affect the process of organogenesis by providing a barrier effect of vascular colateralist during process Angiogenesis. Angiogenesis is a complex process involving of extracellular matrices, proliferation, endothelial cell migration, and functional maturation

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of the new endothelial cells into a blood vessel. When the process of angiogenesis interupted it will efect the distribution of nutrition to the body part of the embryo that can efect the development of body weight and thickness.

2 MATERIALS AND METHOD

The tools used in this research include egg incubator, egg tray, petri dish, Effendorf tube, vortex mixer, scales, organ pots, dispossible 1 cc onemade, dispossible 5 cc Onemade, object glass, cover glass, tweezers, Scissors, egg shell punch, autoclaves, staining jar, masking tape, glove, pipette, camera, digital scales and calipers. The materials used in this study include the unincubated chicken eggs, turmeric rhizome simplisia powder, ringer's lactate solution, NaCl 0.9% liquid and DMSO 100% liquid.

Preparation of the turmeric rhizome simplisia with making a solution of DMSO 2% by dissolving DMSO 100% into the solution of NaCl 0.9%. Turmeric rhizomes simplisia powder as much as 0.48 mg/gr eggs for the treatment group of P1 and 0.96 mg/gr eggs for group treatment P2 diluted with DMSO 2% to reach the volume of 1.25 mL then homogenized using a vortex mixer.

The location of the egg sack was marked by a pencil, as well as on the top of a egg sack for the spot to punch the eggs. The next step was to punched the egg as wide as the syringe needle in the marked part and then injected the turmeric rhizome simplisia as much as 0.25 mL in the P1 group and as much as 0.25 mL in the P2 group. The hole where was the injection done than be closed using masking tape and the egg labeled.

The incubation of eggs was done in incubators with a temperature of 38°C for 72 hours then carried out the injection of the turmeric rhizome simplisia and incubated at the temperature of 38°C until the embryo reaches the 10th days old. The position of the egg in the incubation was vertically with the egg sack located above.

The egg shells are broken down by knocking the egg sack using tweezers without cange the position of the eggs. The embryo from the egg caried out into the petri dish then the embryo are taken and soaked in ringer's lactate solution while shake several times to remove the remaining egg yolks. A clean embryo was measured the body weight by digital scale and the thickness by calipers.

The body weight was measured to within 0.1g using electronic scales while the body width was taken across the widest point of the abdomen in embryos (Browne, 2006).

3 RESULT

The results showed that the turmeric rhizome simplisia at dose of 0,48 mg/gr caused died in one of incubated chicken egg and at dose of 0.96 mg/gr caused two of five incubated chicken egg was died. The factor that caused died in incubated egg with inoculation of trumeric rhizome simplisia was not known exactly, however, the embryos have been death when we hasvest it. We suggest that apoptosis is probably a cause of death of chicken embryos that was inoculated with turmeric rhizome simplisia. Piwocka et al (2001) said that curcumin a yellow pigment from Curcuma longa, curcumin may induce apoptosis in normal and cancer cells, it induces nonclassical apoptosis via a still-unrecognized mechanism, which leads to chromatin degradation and high-molecular-weight DNA fragmentation. In the other hand, research that conducted by Chen et al (2010) showed that curcumin induces apoptotic injury effects on mouse blastocysts through ROS generation, and further promotes mitochondria-dependent apoptotic signaling processes to impair sequent embryonic development. The left eggs from group of dose 0.48 mg/gr (P1) and 0.98 mg/gr (P2) have decreased of body weight and thicness compared with negative control group (K-) (Figure 1 and 2).

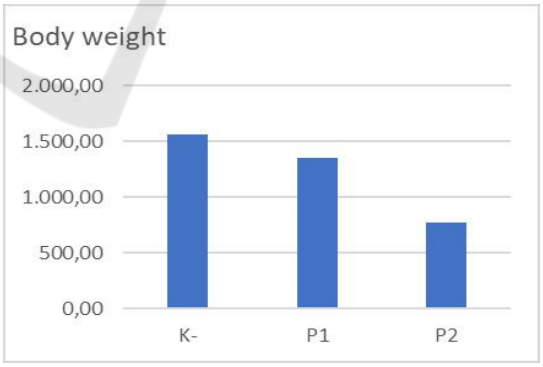

Figure 1. Body weight of Chicken Embryo Inoculated with Turmeric Rhizome Simplisia and Incubated for 10 Days.

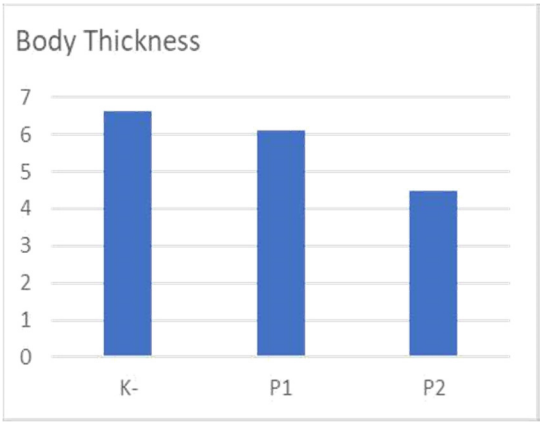

Figure 2. Body thickness of Chicken Embryo Inoculated with Turmeric Rhizome Simplisia and Incubated for 10 Days.

4 DISCUSSION

Weight loss can be used as an indicator of abnormalities at the time of the organogenesis phase. The organogenesis phase involves the nutrients, oxygen, and disposal of residual metabolic substances. These needs can not be covered if only from diffusion resource so that there must be a new system for embryo development. It encourages the occurrence of angiogenesis. The process of angiogenesis also occurs in the process of adipogenesis. Adipogenesis involves two processes the formation of precursor adipocytes known as preadipocytes of stem cells mesenchyme and differensiation preadiposit become a mature and functional adipocyte. The functionalization adipocyte cells continue to develop and in its development requires nutrients and oxygen to form blood vessels through the process of angiogenesis (Billon et. al., 2010).

The body weight and thickness of the chicken embryo are closely related with grwoth of the adipose tissue. According to the Ejaz et al (2009) the growth of adipose tissue is similar to the growth of cancer that requires new blood vessels. In the adipose tissue, this angiogenesis is mediated by adipokines which includes leptin, adiponektin, aesistin, visfatin, TNF-α, IL-6, IL-1, and VEGF. The curcumin in turmeric rhizome simplisia can inhibit adipokines which induces angiogenesis. In addition to inhibiting Adipokin, curcumin inhibits the expression mRNA VEGF. In the process of adipogenesis, curcumin decrease its differentiensiation of preadipocytes so that the amount of mature and functional adipocyte also decline. According to Gonzalez-Castejon and Rodriguez-Casado (2011) curcumin regulate the genes involved in energy metabolism and the accumulation of lipidas well as lowering intracellular lipid levels. The ability of curcumin to decrease the VEGF levels that affect the decrease in blood numbers also affects angiogenesis in adipose tissues that adipose tissue does not develop and causes reduces body weight and thickness. We suggest that the mechanism of curcumin that inhibit angiogenesis and adipokines give effect to decrease body weight and thickness of the embryo.

5 CONCLUSION

Induction of curcuma longa simplisia in chicken embryo incubated 10 days was showed give efect to the body weight and thicness, even it just a small effect.

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