

PHYSIOLOGICAL TOLERANCE TEST OF COMBINATION TREATMENT OF ANTIGEN-G AND CURCUMIN EXTRACT ON VEGF LEVELS, MORTALITY RATE OF BALB/c

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Abstract

The paper aimed to present the Physiological Tolerance Test of Combination Treatment of Antigen-G and Curcumin Extract on VEGF Levels, Mortality Rate of BALB/c. This research consists of several stages. The TmD-G antigen (ITMd) was isolated from the thoracic segments of Musca domestica larvae 4th instar stage and the thoracic segments of the adult cycle stage, 5 days old. The larvae were obtained from the rearing process using defined media. Curcumine extract (EK) was obtained from Curcuma xanthorrhiza rhizomes through the process of broyage, mixing, precipitation, lyophilization and collection of dry EK. The VEGF serum molecules were significant higher ($P < 0.05$) in Ek20Ag3 to Ek20Ag9 then in other combination treatment. The zero-mortality rate obtained in using AgLMd.

Key words: antigen, curcuma extract, insect, VEGF.

INTRODUCTION

The availability of abundant local natural resources in Indonesia has great potential to support the production of medicinal raw material technology. The natural resource in this basic research is a combination of natural G-antigen from *M. domestica* and curcumine as an angiogenesis controlling substance to overcome the problem of hypoglobulinemia and abnormal angiogenesis in neonatal mammals. Simultaneously, this research has great potential to be used as a national bio-pharmaceutical ingredient sourced from TmD antigen which has immuno-enhancer power in improving the synthesis of IgG antibodies and anti-microbial substances that function to control angiogenesis when organ tissue damage occurs. This research is a basis for improving neonatal livestock immunity in order to support national food security which in turn has an impact on national economic recovery.

Various studies conducted on mammalian livestock show that cases of failure of passive transfer of immunoglobuline are often experienced by neonates (Staněk et al., 2019; Weström et al., 2020) so there is an urgent need to increase immunoglobulin (Stockler et al., 2021) which allows neonates to maintain their lives (Rumokoy et al., 2016).

A common clinical feature of symptomatic hypogammaglobulinemia is a predisposition to infection that should be countered by an antibody response (Bussel et al., 2021). Damage to cells such as in the lungs and other organs including the digestive tract caused by viral, bacterial or tumor infections can result in hypoglobulinemia and reciprocally pathogenic microbial infections can result in hypoxic conditions and cause severe hypoglobulinemia (Cekic et al., 2020). Damage to cells in neonates can reduce oxygen supply and increase VEGF as an angiogenesis factor (Howell et al., 2013) (Figure 1).

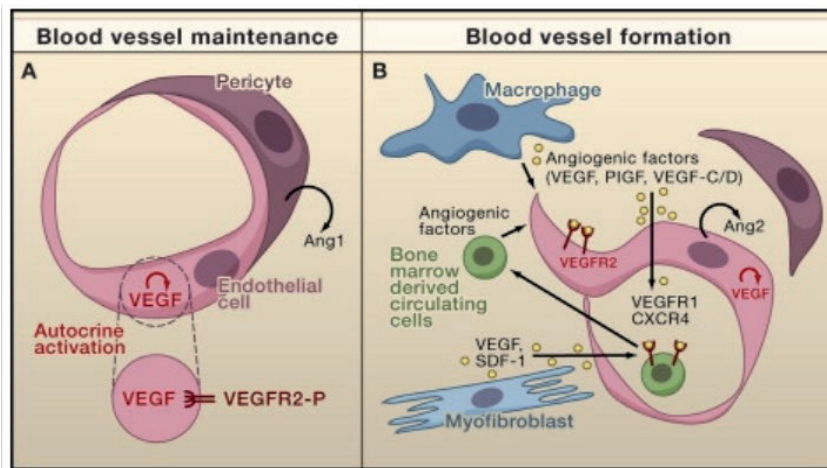


Figure 1. The role of VEGF in blood vessel maintenance and blood vessel formation (source: Heloterä & Alitalo, 2007)

Cunningham-Rundles (2019) described the most common human immunodeficiency encountered in clinical practice is a congenital immune defect. Immuno-therapy and control of angiogenesis using insect antigen biopharmaceuticals combined with curcumin simultaneously in cases of immune deficiency or hypo-globulinemia and abnormal cell regeneration is a new hope that has never been studied and is still a bright hope. Although there are no research reports regarding the combination of two antigen immuno-enhancing substances Ag-TM_d and curcumin to treat cases of hypoglobulinemia, angiogenesis abnormalities and regeneration of cells damaged by virus infection, several previous scientific reports have begun to indicate several different potentials of insect antigens and curcumin. Gong et al. (2012) tested the role of insect immunogen protein as an adjuvant that has the potential to be used as a vaccine candidate in overcoming influenza virus infection. Other scientific reports show that the salivary glands of flies from the species *Haematobia irritans* can be used for the immune system of mammalian organisms (Breijo et al., 2018). Toar et al. (2019) stated that the use of insect antigen from *Apis mellifera* as a source of antigen stimulated the production and circulation of antibodies in traditionally reared goats.

MATERIALS AND METHODS

This research consists of several stages. The larva antigens (LTMD) were isolated from

the thoracic segments of *M. domestica* larvae instar IV, while the antigens TMD_d were isolated from adults of this insect of 5 days old. The larvae were obtained from the rearing process using defined media (Figure 2).

Curcumin extract (EK) was obtained from fresh *Curcuma xanthorrhiza* rhizomes through a process: collection, selection and *broyage*, mixing, precipitation, lyophilization and conservation. Larvae from 1st instar (DOL) were placed and reared in a container until 4th instar. The rearing box with dimensions (30 x 30 x 30 cm) by placing it four containers with a diameter of 15 cm and a height of 8.5 cm.

The larvae were fed with a *defined media*. The thoracic immunogen extract (Ag-TMD_d) followed the TMD_d antigen extract procedure for both larvae and adults. The crude extract preparations of thoracic immunogen LM_d and AM_d were filtered using a micro filter (0.22 μm) obtained from Labbox Labware Vilassar de Dalt Barcelona Spain and were sterilized with UV light for 12 hours, to avoid micro-organism contamination. UV light was carried out using T5 Ultra Violet Sterilization. The LTMD antigen serum extraction procedure follows the ATMD procedure. Detection of the value of the proportion of antigen-G using a photometric (Brix%). The physiological test of combination treatment of Antigen and curcuma extract on mortality rate were calculated based on the percentage of animal died. The data of LTMD and ATMD were analysed with t-test, while the data of VEGF level were analysed the variance (Anova).

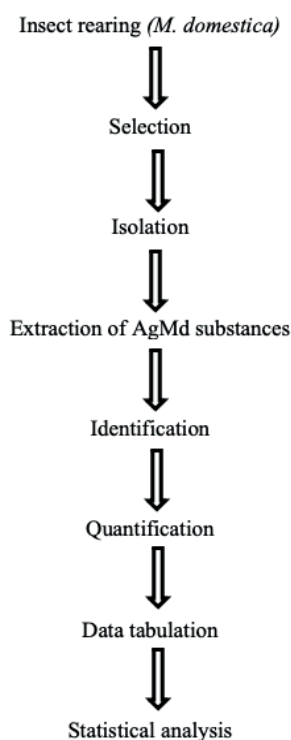


Figure 2. The main stapes in AgMd proportion quantification

RESULTS AND DISCUSSIONS

Comparison of antigens proportion value obtained from LTMD and TDMd extract as shown in Figure 3 was non significant difference ($P > 0.05$). The average value of the LTMD antigen is 3.8 which tended to be higher than the average value of TDMd which reached 3.73. The highest figure for AgLTMD is 4.33 while AgTDMd was 4.67 Brix% slightly higher than in LTMD. The similarity in the concentration of these antigens concentration indicated that these substances have the same opportunity to be used in supporting the active immune system in livestock, although the synthesis of antibodies in mammals was always oriented towards the presence of antigens that will be specifically recognized by antigen receptors which will then proceed to the mechanism of immunity for synthesis of antibodies which specifically has the ability to recognize and destroy the related antigen structures in the body. On the other hand, we are also interested in exploring later the chemical composition of the antigens present in both sources (AgLTMD and AgTDMd).

Several studies reported that insect antigen extracts have a potential to improve the immune system of livestock (Ameri et al., 2008; Toar et al., 2017; Coup et al., 2004) and be used as an

alternative strategies to overcome insecticide resistance (Oyarzún et al., 2008) while controlling the negative influence of insect as pest in cattle farm. These results still need to be continued by exploring the role of immun-enhancers obtained from LTMD and TDMd: at which stage in the mechanism of immunoglobulin-G synthesis supports the increase in blood serum IgG levels.

The combination treatment of AgTMD and curcuma extract on concentration level vascular endothelial growth factor of BALB/c shown in Figure 4. The VEGF serum molecules were significant higher ($P < 0.05$) in Ek20Ag3 to Ek20Ag9 then in other combination treatment. This performance indicated that the combinations treatment with Ek40 and Ek60 gave a positif effect in reduction of BALB/c VEGF serum. This molecule plays a role in pathological angiogenesis associated with various cases of tumor growth and metastasis (Melincovici et al., 2018). According to Ferrara et al. (2004) the condition of hypoxia in an individual could be the main trigger for the synthesis of VEGF to promote the blood vessel formation (Goodsell, 2003). In this condition where the tissue is damaged, for example a 'wound' occurs, VEGF can help to form blood

vessels in the location where is undergoing healing from the damaged cells.

Table 1 presented that the combination using AgL-Ek treatment caused a zero-mortality rate.

We found a 12.5% of mortality rate in groups of combination of AgA-Ek but there was another accident in cage that caused this mortality (Table 2).

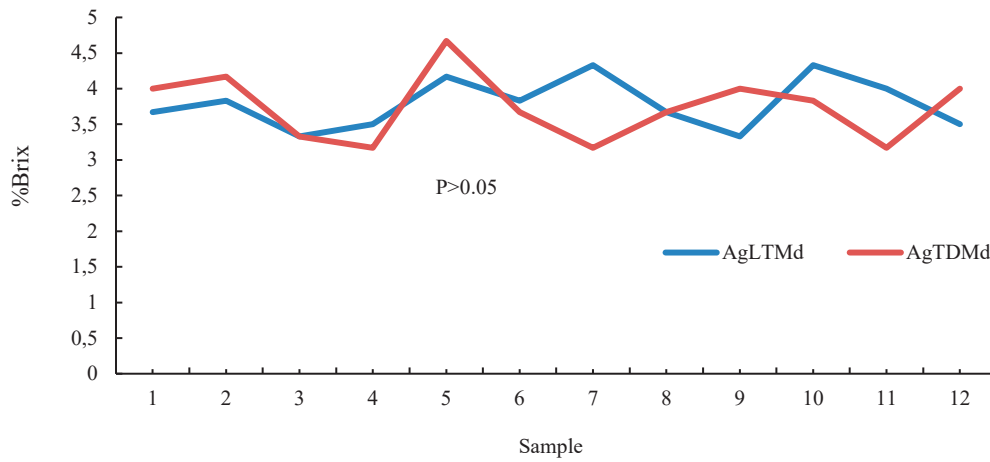


Figure 3 Comparaion of Antigens Proportion value of AgLTMD and AgTAMd

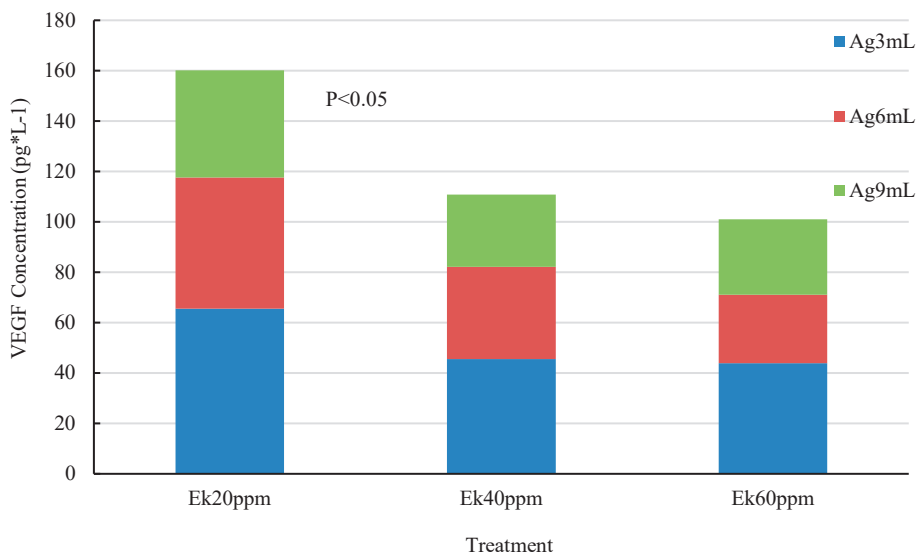


Figure 4 Graph of VEGF Concentration of Experimental BALB/c

Tabel 1. Combination treatment of AgLMd-Ek on Mortalitas Rate

Treatment	Ek40 ppm	Ek60 ppm
AgLMd 3mL	0	0
AgLMd 6mL	0	0
AgLMd 9mL	0	0

Tabel 2. Combination treatment AgAMd-Ek on Mortalitas Rate

Treatment	Ek40 ppm	Ek60 ppm
AgAMd 3mL	0	0
AgAMd 6mL	12.5	0
AgAMd 9mL	0	0

CONCLUSIONS

The use of antigen extract of AgMdinsect up to 60 mL and curcuma extract up to 60 ppm tended to be a safe substance, while this level of curcuma have a positive effect to reduce the VEGF molecules in BALB/c serum

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