Scientific Papers. Series D. Animal Science. Vol. LXVI, No. 2, 2023
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

IMMUNOGLOBULINE-G LEVEL AND BODY WEIGHT OF BALB/C RECEIVING COMBINATION TREATMENT OF LYOPHILIZED *Curcuma longa* AND *Curcuma xanthorrhiza* DURING AN ANGIOGENESIS EXPERIMENTAL

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Abstract

*Immunoglobuline synthesis is largely determined by the presence of the type of antigen that enters the body. However, various biological bioactive substances have a potential to boost individual immunity processes. In this study, 5 weeks old BALB/c was used. This study aims to evaluate the role of the combination of Curcuma xanthorrhiza and Curcuma longa lyophilisate on serum immunoglobuline-G level, angiogenesis process and BALB/c body weight. The bio-active substances were distrubuted through drink water. This study used a randomized complete design arranged with factorial 2*4. The results showed that treatment had no significant effect on IgG levels and the process of angiogenesis (P>0.05), but had a significant effect on body weight (P<0.05). We concluded that combination of Curcuma xanthorrhiza and Curcuma longa lyophilisate could be used in wound healing during an angiogenesis process.*

Key words*: BALB/c, Curcuma, immunity, liopylisate.*

INTRODUCTION

Excellent health in animals is one of the key determinants of being able to produce and reproduce optimally. Health problems could disrupted a biochemical processes in tissues which negatively affected various body organs to carry out systemic physiological functions.

If an organ or part of the body is damaged, for example in the form of injury, especially to a muscle tissue, repairs needed to the cells of that tissue (Wilkinson et al., 2020; Marin et al., 2017) through the process of angiogenesis (Sorg et al., 2017; DiPietro et al. , 2016).

According to Li et al. (2003), angiogenesis at normal levels will play a role in cell regeneration to heal tissue damage in the wound area, especially open wounds on body surface which having a potential to become an 'entrance' for pathogenic microbes or infections (Leapar et al., 2015) so strategies are needed through appropriate treatment (Veith et al., 2019).

An individual's immune condition is very needed to protect body from a threat of microbes infectious (Yu et al., 2017; Cunningham, 2019; Goenka & Kollmann, 2015).

The individual immunity of young mammalian animals is highly dependent on the success of obtaining maternal antibodies contained in colostrum (Toar et al., 2019; Lewis et al., 2017; Hanson et al., 2007). This mechanism is made possible by the natural passive transfer process of antibodies (Rumokoy et al., 2017), becoming an important problem if this process fails (Staněk et al., 2019).

Mother colostrum antibodies contain many IgG antibodies which are widely used for human and animal health (Arslan et al., 2021; Stockler et al., 2021).

As time increases, day by day, the mammalian body will easily synthesized antibodies (Goenka & Kollmann, 2015). The level of ability to synthesize some types of antibodies depends on various factors such as environmental conditions (Amasawa et al., 2021) and nutritional intake, various bio-molecules that enter the body either through drinking water or through food as well as the type of antigens

exposed to the body (Toar et al., 2021; Cuop et al., 2004).

Various scientific reports showe that various bio-molecules from plants have a significant role in helping body to synthesize antibodies (Weström et al., 2020; Rumokoy et al., 2016; Breijo et al., 2018; Toar and Rumokoy, 2021).

The *Curcuma longa* plant is a natural resource which is mainly known as a kitchen spice. Apart from that, the rhizomes of this plant are widely used in traditional medicine in various regions to help maintain body stamina in relation to endurance (Rosidi et al., 2014). Another type of plant that is similar to *C. longa* is *Curcuma xanthorrhiza* which was used for various health benefits. This substance has a great potential in controlling various threats of pathogenic microorganisms and parasites in traditional livestock farming which has recently received a lot of attention through research activities, including being able to control the synthesis of VEGF in overcoming angiogenesis abnormalities (Melincovici et al., 2018; Rumokoy et al., 2023).

MATERIALS AND METHODS

This study used 24 neonates of five weeks old experimental BALB/c mice; lyophilized extract of *Curcuma longa* and *Curcuma xanthorrizha*; rearing box with dimensions 30 cm in length, 20 cm in width and 20 cm in high, each cage was placed four mice.

The cages were equipped with a feeder and nippled drinking bottle. The others main materials used in this research were: measuring cup, petri-dish, micro-tube, micro-pippet, alcohol (70%), MDP digital scale max. 500 g; wide rolls of tissue; gel plate, radial immuno-diffusion plate, 0.1 M buffer solution pH 7, 0.1% nitric acid, 1 µg/ml amphotericin B, 0.002 M ethylene-diamide-etheric tetraacid and serum immunoglobulin for antibody analysis. AD1 of commercial feed (70%) and grounded yellow corn were mixed and used as mice feed to this experiment.

The variables observed were: total of serum immunoglobulin-G (IgG); tissue healing and body weight. The quantity of serum antibody total was detected through a single radial immuno-diffusion (SRID) test. The IgG blood sample serum was obtained by using a technic of tail blood collection.

This step of wound healing was evaluated during the angiogenesis which measured the percentage level of epidermal tissue recovered after a wound done purpose in tail base (TB) of mice.

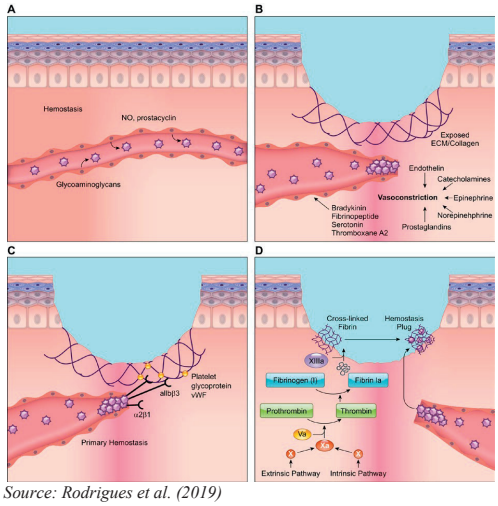
The final body weight of the experimental animals was measured after two weeks of experiment at seven weeks old.

The material of fresh *C. xanthorrhiza* (Cx) and *C. longa* (Cl) were purchased from local agriculture production then its rhizomes were selected and cleaned to get the best rhizomed.

The next steps were *broyage*, mixing, and then precipitation. Dry lyophilization were proccesed for about two weeks before using.

This study used a completely randomized design arranged by factorial A*B (2x4), factor A was the level of *C. longa* (A1 0 $mg*L^{-1}$ and A2 5 $mg*L^{-1}$) and factor B was the level of *C. xanthorriza* as immuno-enhancer per litter of drinking water: B1 0 $mg*L^{-1}$; B2 5 $mg*L^{-1}$; B3 10 $mg*L^{-1}$; B4 15$mg*L^{-1}$

The data were statistical analysis to evaluate the significance effect of treatment by using SPSS software.



Source: Rodrigues et al. (2019)

Figure 1. The process in wound healing

RESULTS AND DISCUSSIONS

The results of this study were presented by the following figures.

Figure 2 presented the effect of combination combination of *C. longa* and *C. xanthorrhiza* on IgG serum level of BALB/c. We found an significant interaction in this experiment ($P<0.05$).

The treatment Cx15-Cl15 caused an highest effect on IgG serum level (172.75 mg IgG in 1 dl of blood serum) followed by effect of treatment of Cx-Cl15 that reached 181.25 IgG in blood serum while the lowest response shown in Cx0-Cl15 treatment obtained in 165 mg of IgG in a litter of serum.

These responses indicate the important role of these combination substances in supporting the body's defense against infection in its environment.

This effect could be caused by the role of immunoregulators and neuromodulators of the susbtances of curcumin in animals when exposed to pathogenic agents that lead to the immune system, especially in IgG synthesis as related to the scientific report of Szelényi (2001). Widjaja et al. (2022) has shown a modulator effect of curcumin on boosting the immunity of people received a COVID-19-vaccination.

A non significance effect of a combination treatment of lyophilized *C xanthorriza* and *C. longa* ($P>0.05$) on tissues healing of BALB/c shown in Figure 3. Tissue healing depends more on balanced nutritional intake (Timms, 2011). An epidermal growth factors, stem cell therapies could be an aliternative solution in tissues healing (Vyas and Vasconez, 2014). The steps of wound healing presented by Rodrigues et al. (2019) as show in Figure 1.

The highest response tended to dominate in the treatment of combination of Cx15-Cl10 (15 mg *C. xanthorrhiza* per 1 litter of drink water combined with 10 mg *C. longa* per drink water). These results indicate that the application treatment level of bioactive substances in this study from the two types of curcuma does not have a significant role in the angiogenesis process for tissue repair.

Another possibility is that the size of the damaged tissue area in this experiment did not involved the function of the curcuma used.

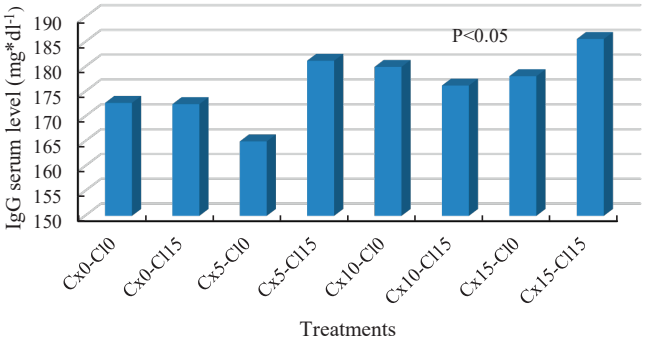


Figure 2. IgG Level of BALB/c

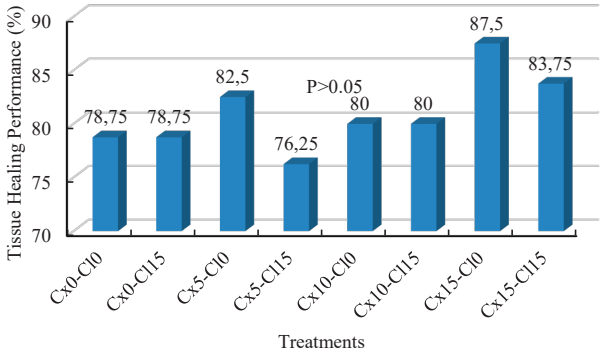


Figure 3. Treatment Effect on Tissues Healing

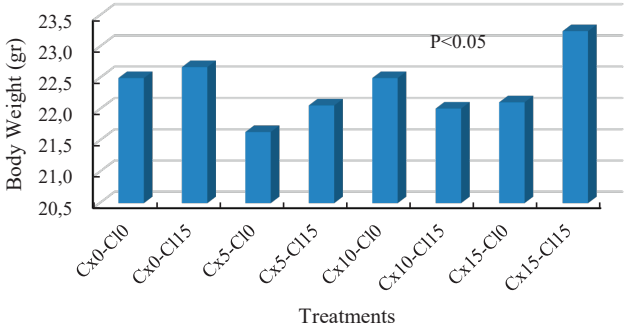


Figure 4. Treatment Effect on Body Weight of BALB/c

The Figure 4 represented significance effect of combination treatment of lyophilized *C. xanthorriza* and *C. longa* ($P<0.05$) on body weight of BALB/c after two weeks of treatment. The highest response was found in the treatment of combination of Cx15-Cl15 (15 mg *C. xanthorrhiza* per 1 L of drink water combined with 15 mg *C. longa*). This results related to the report Al-Sultan (2003) that an utilization 5% of *C. longa* in diets could increased a body weight. The curcuma contained antioxidant that has an important role for body protection to ensure biochemical processes in the body.

CONCLUSIONS

The treatment of combination substances of *Curcuma longa* and *Curcuma xanthorrhiza* has a positive effect on IgG antibody level and body eight gain in drink water of BALB/c.

ACKNOWLEDGEMENTS

The authors fully expressed they gratitude to Sam Ratulangi University: This *R_MAPALUS* realization was financed by BLU-PNBP 2023 - DIPA of Sam Ratulangi University *(No: 1398/UN12.13/LT/2023)* through LPPM Unsrat in Academic Year of 2023.

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