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Submission date: 14-Mar-2023 12:27PM (UTC+0700)

Submission ID: 2036807139

File name: 4._JAPTR-12-109.pdf (1.43M)

Word count: 2485

Character count: 13360

Egg white-induced inflammation models: A study of edema profile and histological change of rat's paw

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J. Adv. Pharm. Technol. Res.

ABSTRACT

The egg white was used to induce rat paw inflammation, with inadequate references to explain its mechanism. It's contained protein was identified as an allergen was suspected to trigger an inflammatory reaction. This research was aimed to evaluate the use of egg white as an inflammatory inductor in inflammation animal models through edema profile and histological change. Male Wistar rats were divided into three groups, which were given λ -carrageenan, fresh takes of the hen's egg white, and sterile saline solution. Edema was induced by subcutaneous injection of 0.1 ml of λ -carrageenan (1%), egg white, and sterile saline solution as the control in the hind paw of rats. Paw volume was measured before and then at 1, 2, 3, 4, 5, 6, and 24 h after the inductor injection. Paw tissue was taken for evaluation of rats' paw histological change. The data were analyzed by one-way ANOVA followed by LSD test. The results of the study showed that the egg white could induce rat paw inflammation. Edema formation began in the 1st h and reached the peaks in the 2nd h after the subcutaneous injection of egg white. A number of leukocyte cells were also found in the inflamed paw tissues. Egg white was potential as an edema inductor for animal models of inflammation for the evaluation of new drugs or natural product with anti-inflammation activity.

Key words: Animal models, edema profile, egg white, histological change, inflammation

INTRODUCTION

Inflammation was the body's response to harmful stimuli such as pathogenic infection or tissue damage.^[1,2]

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Submitted: 29-Nov-2020

Revised: 08-Feb-2021

Accepted: 12-Mar-2021

Published: 27-Apr-2021

This response occurs in two phases, acute and chronic inflammation with each characteristic.^[3] Various models of evaluation of drugs or natural products with anti-inflammatory activity were developed refers to the characteristics of the inflammatory phase. Common methods were used for evaluating anti-inflammatory activity are based on the ability of the compound to inhibit edema after injection of the inflammatory agent in the rat's hind paw.^[4]

Carrageenan was widely used as an inductor of inflammation, with a clear mechanism of induction of an inflammatory response.^[5] Carrageenan induced rat paw edema through two phases. Early phase mediated by histamine, serotonin, and increased local synthesis of prostaglandin occurred

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How to cite this article: Barung EN, Dumanauw JM, Duri MF, Kalonio DE. Egg white-induced inflammation models: A study of edema profile and histological change of rat's paw. *J Adv Pharm Technol Res* 2021;12:109-12.

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1–2nd h after induction, then later phase (3rd h) mediated by bradykinin, leukotrienes, leukocytes infiltrations, and biosynthesis of prostaglandin by inducible cyclooxygenase.^[6-8]

Other researchers used fresh taken of the egg white to induce rat paw inflammation,^[9-11] with inadequate references to explain its mechanism of edema formation. The egg white contains protein such as ovalbumin (54%), ovotransferrin (12%), ovomucoid (11%), ovomucin (3,5%), and lysozyme (3,5%).^[12] These proteins have been identified as the major allergen in egg white,^[13] which, if it was injected into the rat's paw, suspected to trigger an inflammatory reaction.

This research was aimed to evaluate the use of egg white as an inflammatory inductor in inflammation animal models through edema profile and histological change. The result can provide preliminary data on inflammatory mechanisms in egg white-induced inflammation animal models.

MATERIALS AND METHODS

Materials

λ -Carrageenan was purchased from Sigma-Aldrich. Hen's egg was purchased from the local market in Manado, North Sulawesi (Indonesia).

Determination of protein content in hen's egg white

The determination of protein content in hen's egg white was performed at the Balai Riset dan Standardisasi Industri Manado using the methods from Indonesian National Standard (SNI) number 01-2891-1992.^[14]

Animals

Male Wistar rats (150–200 g) were obtained from the Laboratory of Pharmacology, Department of Pharmacy, Poltekkes Kemenkes Manado. The protocol of animal experiments was approved by the Health Research Ethics Committee Poltekkes Kemenkes Manado with approval number KEPK/01/04/2020. All animal experiments were divided into three groups, each consisting of 8 rats.

Research design

Carrageenan- and egg white-induced rat paw inflammation

Edema was induced by subcutaneous injection of 0.1 ml of 1% λ -carrageenan (in 0.9% sterile saline solution) (Group I)^[15,16] and 0.1 ml of fresh takes of the hen's egg white (Group II) in the hind paw of rats.^[9-11] The third group served as a control group, which were injected with 0.1 ml sterile saline solution. Paw volume was measured by digital plethysmometer (Almemo® 2450, Ahlbom) before (0 h) and then at 1, 2, 3, 4, 5, 6, and 24 h after the inductor injection.

Histopathology of paws

For histopathology examination, rats were sacrificed under anesthesia conditions. Paw tissue of all groups was taken and then fixed in 10% buffered formalin and stained with

hematoxylin and eosin to the evaluation of inflammatory change in the rat's paws.

Statistical analysis

The data about the difference of paw volume were presented as mean \pm standard error mean ($n = 8$) and analyzed by one-way ANOVA followed by LSD test using SPSS software (IBM SPSS Statistics ver. 25). $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

The present study is expected to describe the utilization of egg white as an inductor of inflammation and its possible mechanism. Hen's egg whites were used in our study contained 10.51% protein (Analysis Number 101/1/PT/LB/VII/2020), consistent with the previous study was showed that the egg whites contain 9.7%–10.6% of protein.^[17] Egg white was content allergens protein,^[13] was suspected to contribute to edema formation. However, egg white contained proteins with unique pro- and/or anti-inflammatory properties^[18] and therefore need further research to determine the protein fraction that plays a role as an edema inductor.

The subcutaneous injection of λ -carrageenan and egg white significantly induced rat paw edema and no signs of edema were detected in the paw of the control group [Figure 1]. To assess histologically change during the inflammation process, rat's paw tissue from all group were examined with hematoxylin and eosin stain. The edema formation and infiltration of the leukocyte cells were detected in inflamed rat's paw tissue due to carrageenan and egg white induction, compared with the control group [Figure 2].

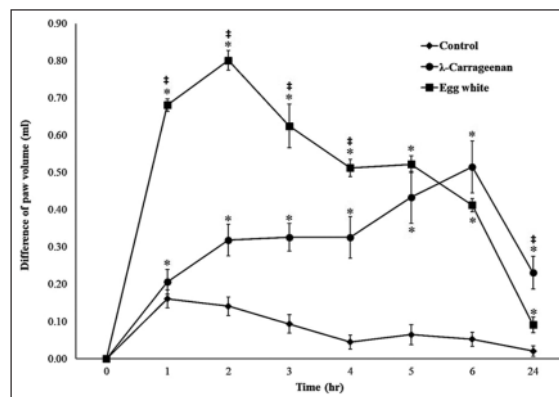


Figure 1: Paw edema of carrageenan- and egg white-induced inflammation rat's paw. Values are mean, $n = 8$. *Indicated significance level with the control group and †Indicated significance with carrageenan group

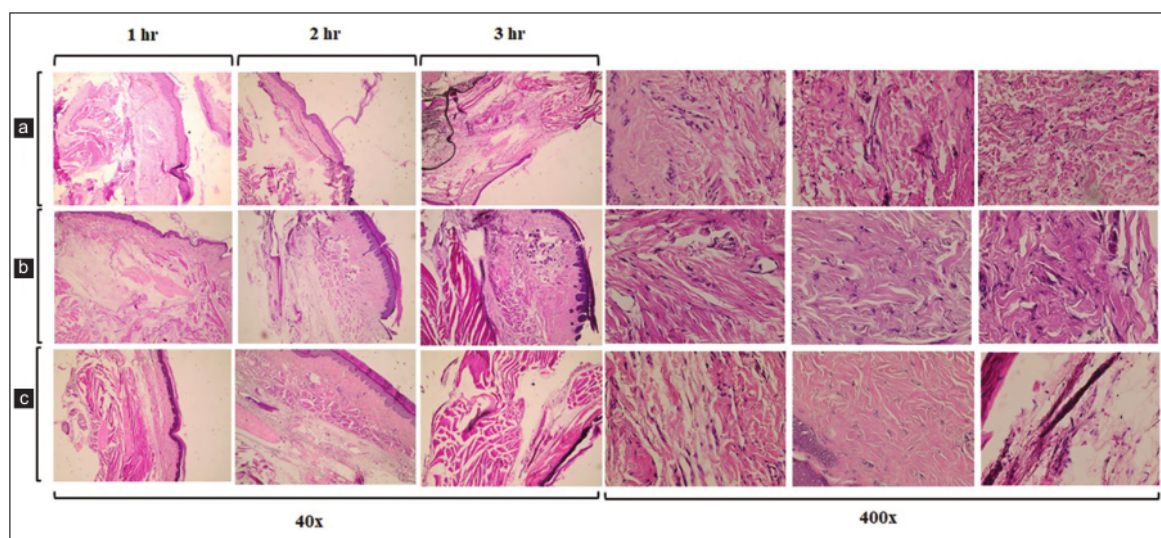


Figure 2: Histological data of paw tissue of carrageenan (a), egg white (b), and control (c) treated rats after stained with hematoxylin and eosin

The carrageenan-induced paw edema models were widely used for the evaluation of the anti-inflammatory activity because it has the advantage of not causing damage to the injected tissue.^[5,19] The present study showed edema formation and leukocyte infiltration in inflamed paws after injection of carrageenan [Figures 1 and 2] with a clear mechanism was described above. The previous study has reported that the found a large number of leukocyte in paw tissue after injection of carrageenan^[20,21] and there was a positive correlation between increased leukocyte infiltration and edema formation.^[22]

The results of the study showed that the egg white could induce edema of the rat's paw. Edema formation began in the 1st h and reached the peaks in the 2nd h after the subcutaneous injection of egg white [Figure 1]. A number of leukocyte cells were also found in the inflamed paw tissues [Figure 2]. Possible mechanism of egg white induced edema mediated by released histamine and serotonin.^[19,23] The previous study has shown that the peak of edema in rat's paw has occurred in 1 h after an injection of histamine,^[24] while the edema curve due to injection of serotonin has increased time-dependent manner (until 3 h).^[25] During the acute inflammatory response, histamine and serotonin were the main mediators that caused the increased vascular permeability and edema formation, also migration of leukocyte cells.^[26,27] We also found that at 5th and 6th h, the difference of paw volume of egg white-induced edema was not significantly with carrageenan-induced models [Figure 1]. For this mechanism of edema formation, more research is needed.

CONCLUSION

Egg white was potential as an edema inductor for animal

models of inflammation for the evaluation of new drugs or natural product with anti-inflammation activity. To clearly inflammatory mediators involved during the edema formation due to egg white-induced inflammation, further research is needed.

Acknowledgment

The authors would like to express their gratitude to Poltekkes Kemenkes Manado for financial supported through Risbinakes 2020 scheme.

Financial support and sponsorship

This study was financially supported by Poltekkes Kemenkes Manado by Risbinakes 2020 scheme.

Conflicts of interest

There are no conflicts of interest.

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