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Combined Effects of Noni Fruit Extract (*Morinda Citrifolia* L.) and Warfarin on Bleeding and Coagulation Time of Mice

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Research Article

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Abstract

Background and Objectives : Noni (*Morinda Citrifolia L.*) fruit has been studied for its platelet antiaggregation effect. Warfarin is one of the synthetic derivative of coumarin compounds, which is regulary use as an oral anticoagulant. There is a possibility of potentiation platelet antiaggregation activity which is characterized by bleeding and coagulation time. The objectives of this research is to study combined effect of warfarin and noni fruit extract to mice's bleeding and coagulation time.

Methods : Bleeding time was determined through tail bleeding method. Coagulation time was determined through cappillary pipe method. Mice were randomly divided into 3 groups. Group 1 received noni fruit extract 100 mg / kg bw once daily, group 2 received warfarin 1 mg / kg bw once daily, group 3 received warfarin 1 mg / kg bw + noni fruit extract 100 mg / kg bw once daily, each given for 7 days. Bleeding time and coagulation time were tested twice, once before being treated and then on day-7 treatment.

Result : Result of this study showed that bleeding and coagulation time was significantly increased after treatment. Group 1 which received noni fruit extract 100 mg/kg bw, bleeding time increased from 52.75 ± 1.60 seconds to 111.00 ± 4.45 seconds and coagulation time from 60.00 ± 4.01 seconds to 101.25 ± 3.75 seconds. Group 2 which received warfarin 1 mg/ kg bw, bleeding time increased from 56.62 ± 1.71 seconds to 152.88 ± 5.86 second and coagulation time from 60.00 ± 4.01 seconds to 136.88 ± 4.43 seconds. Group 3 which received combination of noni fruit extract 100 mg/kg bw and warfarin 1 mg/kg bw, bleeding time increased from 56.00 ± 1.43 seconds to 236.38 ± 4.24 seconds and coagulation time from 63.75 ± 3.75 seconds to 213.75 ± 8.85 seconds.

Conclusion : Combination of noni fruit extract and warfarin can prolonged bleeding and coagulation time of mice's.

Keywords: *noni fruits extract, warfarin, combined effect, bleeding time, coagulation time*

Introduction

Noni (*Morinda Citrifolia L.*) is a plant of the *Rubiaceae* family. This plant is grown in almost all Indonesian archipelago. Generally, these plant grow wild on the beach, field, forest, or planted in the home yard as a vegetable or medicinal plant[1]. Noni has various healing properties against various degenerative diseases. Certain research publication aknowledge that noni is efficacious for treating atherosclerosis, diabetes, high blood pressure, sore throat, cough, prevent fat absorption and as diuretic agen[2,3]. Noni fruit has been studied for its platelet antiaggregation effect to prevent thrombosis in atherosclerosis[4,5].

Chemical constituents of noni fruit are coumarin, alizarin, morindin, morindon, prokseronin, rubidin, scopoletin, octanoic acid, potassium, vitamin C, vitamin A, terpenoids, asperulosid, caprylic acid, caproic acid, and rutin[6,7,8]. Coumarin has pharmacological activity as an anticoagulant. One of the synthetic derivative of coumarin compounds is warfarin (dikumarol) which used as an anticoagulant[9]. Patients who use herbal products containing coumarin along with anticoagulant drug such as warfarin requires monitoring for signs of bleeding[10].

Considering the similarity of anticoagulant activity between noni fruit extract and warfarin, there is a possibility of potentiation platelet antiaggregation activity which is characterized by prolonged bleeding and coagulation time. This may occur in patients who regularly take warfarin to prevent thrombosis and also simultaneously consume noni supplements for lowering blood pressure or cholesterol. The aim of this research was to study the combined effect of

warfarin and noni fruit extract to bleeding and coagulation time of mice's.

Material and Method

Samples Collection and Preparation

Samples of noni fruits (*Morinda citrifolia* L.) was collected from several area in Bali. Noni fruits washed, dried and powdered. The powder wrapped and stored in a dry place.

Preparation of Crude Extracts

Noni fruit crude extracts prepared by maceration and then concentrated by vacuum rotary evaporator.

Study Design and Procedurs

This research was an experimental, pre and posttest study group. Mice's were randomly divided into 3 groups, each group consisted of 8 mice's. Group 1 received noni fruit extract 100 mg / kg body weight (bw) once daily for 7 days, group 2 received warfarin 1 mg / kg bw once daily for 7 days while group 3 received warfarin 1 mg / kg bw + noni fruit extract 100 mg / kg bw once daily for 7 days. Bleeding and coagulation time was tested on day 0 (before treatment) and on day 7 of treatment.

Bleeding time determined by the tail bleeding method, while coagulation time was determined using capillary method. To determine bleeding time, mice were put into the holder. Mice tail cleaned with 70% alcohol and mice was wounded with a distance of 2 cm from the tip of the tail along the 2 mm with a cutter. Blood was absorbed by attaching a filter paper. Time was measured from the first blood drip until it stops dripping on filter paper. Bleeding time is interval from the first drop of blood drip until it stop dripping[11].

To test the coagulation time, blood samples were taken via sinus orbital using a capillary tube. The capillary tube then etched using glass cutter along the 0.5 cm every 15 seconds once to obtain fibrin threads in the fracture capillary. Coagulation time is time from first blood start dripping until the first fibrin threads appear in the capillary tube[4,11].

Statistical Analysis

Statistical analyses were performed with Analysis of Variance using the Statistical Product and Service Solutions (SPSS) version 17. This statistical analysis is to determine whether there were a differences between treatment group due to the influence of noni fruit extract, warfarin or combined of noni fruit extract with warfarin to the bleeding and coagulation time of mice's using 95% level of confidence.

Results

Three group of mice's, each group consisted of 8 mice's have received different treatment. Group 1 received noni fruit extract 100 mg / kg bw once daily, group 2 received warfarin 1 mg / kg bw once daily, while group 3 received combination of warfarin 1 mg / kg bw and noni fruit extract 100 mg / kg bw once daily, each for 7 days. All group were comparable in bleeding and coagulation time. In the noni fruit extract group, bleeding time was increased from 52.75 ± 1.60 seconds to 111.00 ± 4.45 seconds and coagulation time from 60.00 ± 4.01 seconds to 101.25 ± 3.75 seconds. At the warfarin group, bleeding time was increased from 56.62 ± 1.71 seconds to 152.88 ± 5.86 second and coagulation time from 60.00 ± 4.01 seconds to 136.88 ± 4.43 seconds. Group 3 which received combination of noni fruit extract 100 mg/kg bw and warfarin 1 mg/kg bw, bleeding time increased from 56.00 ± 1.43 seconds to 236.38 ± 4.24 seconds and coagulation time from 63.75 ± 3.75 seconds to 213.75 ± 8.85 seconds.

All data were normally distributed and homogeneous. Statistical analysis result showed that there were significant differences in each treatment group in bleeding time of mice ($P < 0.05$) and coagulation time of mice's ($P < 0.05$). Bleeding and coagulation time in group 3 (combined of noni fruit extract 100 mg/kg bw and warfarin 1 mg/kg bw) showed the higest increase compared to the other treatment group.

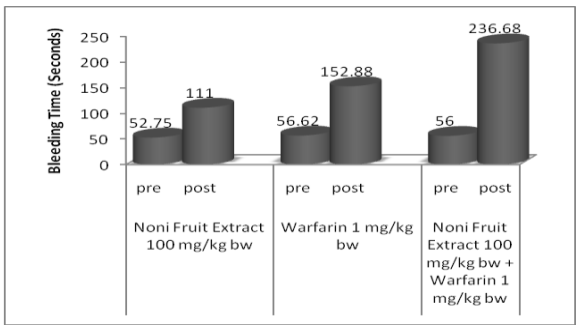

Figure 1. Comparison of Pre and posttest bleeding time parameter

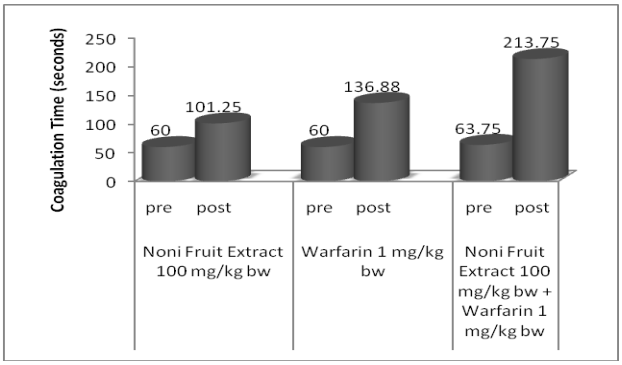

Figure 2. Comparison of pre and posttest coagulation time parameter

Discussion and Conclusion

Bleeding time and coagulation time are two types of dependent variable measured in this study. Bleeding time was observed to see the effect of the treatment to the formation of temporary hemostatic plug which is a hemostatic platelet phase. Bleeding

time is the time required for the formation of a temporary hemostatic plug at the injured area. Prolongation of bleeding time showed that the test compound has effect on coagulation system[4,12,13].

Observations on coagulation time is to see the effect of test compound on secondary hemostatic plug formation, which is the process of hemostatic coagulation[4]. During the coagulation phase, interaction between various enzyme and proenzyme was happen. Activation on one proenzyme, generally forming an enzyme that will activate the second proenzyme and so on in a complex chain reaction. Stage on coagulation phase will change circulated fibrinogen into fibrin which cover the surface of platelet blockage. Platelets are trapped within a highly fibrous structure, forming a blood clot which effectively closed the injured part of vessel. A test compound which can prolonged coagulation time indicates that the compound has an effect on the coagulation process[13].

When two drugs are used together, drug interaction can occur. Drug interaction may increase or decrease the effects of drug. Reduction effect may occur because one of the drugs inhibit the action of other drug. Increasing effect of drug because of drug interaction can be additive or synergistic. Drug interactions are said to be additive if the effect given by the drug combination is same with the sum of the effects of each drug when given alone. Synergistic drug interactions are if effect given by the drug combination is larger (exponential) than the sum of the effects of each drug when given alone. This drug interaction can occur between drugs with other drugs or drug with herbal products[14].

Bleeding time and coagulation time in the group receiving the combination of warfarin 1 mg / kg bw and noni fruit extract 100 mg / kg bw is higher compared to the group receiving a single warfarin 1 mg / kg bw and to the group receiving noni fruit extract only. This shows that there is an additive interaction between warfarin and noni fruit extract. The combination of warfarin and noni fruit extract can prolong bleeding time and coagulation in mice's. This is caused by a common mechanism of action of warfarin and coumarin anticoagulants who are in the noni fruit extract.

Noni fruit extract contains coumarin which is a competitive inhibitor of vitamin K (factor II) in the biosynthesis of prothrombin. Coagulation process requires changes in prothrombin to thrombin. Vitamin K is a cofactor in the conversion reaction. Similarity structure of vitamin K and coumarin can cause compete to bind to the enzyme vitamin K epoxide reductase and vitamin K reductase. It can interfere with the coagulation process is marked by the increasing coagulation time[15].

The combination of warfarin and noni fruit extract could be expected to lead the accumulation of coumarin in circulation and lead to increased activity of coumarin. This study show that combination use of noni fruit extract 100 mg/kg bw and warfarin 1 mg/kg bw can cause increased bleeding time and coagulation time higher than the effect of warfarin or noni fruit extract when given alone. It is need to caution simultaneously use of anticoagulant warfarin with herbal product containing noni fruit extract.

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AUTHORS' CONTRIBUTIONS

Authors contributed equally to all aspects of the study.

PEER REVIEW

Not commissioned; externally peer reviewed.

CONFLICTS OF INTEREST

The authors declare that they have no competing interests.