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# *IN-VIVO* PLATELET ANTI-AGGREGATION ACTIVITY OF CRUDE FUCOIDAN OF *SARGASSUM POLYCYSTUM*

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**Keywords:**

Platelet anti-aggregation, Coagulation time, Bleeding time, Crude fucoidan, ADP, Clopidogrel

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**ABSTRACT:** Platelet or thrombosis is one of the most important factors in blood clots formation. However, activation of platelet aggregation plays a significant role in homeostasis process, but in excess, it causes different cardiovascular diseases such as myocardial infarction, atherothrombosis disease, and coronary artery disease. To reduce the occurrence of these diseases, anti-platelet agents can be used to prevent blood clots formation. Therefore, this study was conducted to test the antiplatelet activity of crude Fucoidan from the brown seaweed (*Sargassum polycystum*) on mice. The measurement parameters used included bleeding time, coagulation time and decrease in plasma uptake by the addition of ADP as a platelet aggregation agent. The mice used were divided into 5 groups: normal control group (0.1% Na-CMC suspension), positive control group (clopidogrel 75 mg/kg), and the crude Fucoidan doses of 50 mg, 100 mg, and 200 mg. The results showed that positive control and crude Fucoidan with doses of 50 mg, 100 mg, and 200 mg groups could prolong coagulation time and bleeding time and also increase the decrease in plasma uptake after ADP addition. Significant differences ($p>0.05$) were also observed with normal controls but positive control of clopidogrel did not show any significant difference.

**INTRODUCTION:** Cardiovascular disease is the highest cause of death in the world. It is caused by the impairment of heart and blood vessels. It is also most popular with coronary heart disease, heart failure, hypertension and stroke [1]. In 2012, ischemic heart disease and stroke alone caused 7.4 million deaths while 6.7 million other people were affected worldwide [2]. Cerebrovascular stroke is the cessation of blood supply to some parts of the brain resulting in loss of function. This can occur due to rupture of blood vessels or obstruction of blood intake to the brain by clots. The postponement in the provision of oxygen and nutrients to the brain causes serious mental problems and in more serious cases, it can lead to death [3-5]. Platelets are usually between 1-4 µm in diameter, oval and flat shaped like a disc. In certain circumstances, they can turn to a round shape with uneven edges due to a bulge called pseudopod.

Their main function is to form mechanical plugs during normal hemostatic responses to vascular wounds [6-7]. This provides many benefits, such as phagocytosis of foreign bodies, and interactions with viruses, bacteria or complex antibody antigens for the organism. However, formation of blockage in these platelets can also be dangerous, for example, it causes thrombosis and embolism which

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can result in atherosclerosis and also increase risk factors for cardiovascular diseases [8]. The formation of blood clots (thrombus) in the arteries can be prevented medically by antiplatelet agents. These drugs are used to reduce platelet aggregation and inhibit thrombosis. Clopidogrel, which is one of these drugs, is a prodrug with one of its metabolites working as a platelet aggregation inhibitor. It selectively subdues binding of adenosine diphosphate (ADP) to platelet P2Y12 receptors and activation of subsequent glycoprotein mediation of MCC/IIIa, thereby suppressing platelet aggregation [6]. However, using this drug also has some side effects including abdominal pain, diarrhea, bleeding (including gastrointestinal and intracranial bleeding [7].

Based on this background, a study on anti-platelet aggregation activity of *Sargassum polycystum* brown seaweed was carried out *in-vivo*. This was done by testing male DDY mice. The parameters examined include bleeding time, coagulation time and decreased plasma uptake induced by ADP. The treatment group was divided into five, each negative control group was given 0.1% Na CMC suspension solution; the positive control group was given Clopidogrel 75 mg/kg while the remaining three groups were administered crude Fucoidan at a dosage variation of 50 mg/kg, 100 mg/kg, and 200 mg/kg respectively.

**MATERIALS AND METHODS:** Crude Fucoidan was gotten from the brown seaweed (*Sargassum polycystum*), ADP (Adenosine difospat), 0.1% Na-CMC, 75 mg/kg BB Clopidogrel, Sodium Citrate, NaCl, 70% ethanol and Aquadest were all used in the experiment.

***In-vivo* Platelet Aggregation Assay:**

**Animals:** The test animals used in the experiment were male DDY mice weighing 20-30g each. Five of them were selected as treatment group while others were further divided into 5 groups weighing 20-30g each. The mice used were active and healthy, characterized with clear red eyes and normal fur. They were tagged using a picric acid solution to distinguish each treatment group. Mice were regularly weighed to control body weight. The experimental protocol was approved by Bogor Agricultural University (Animal ethical Protocol number: 0726/UN2.F1/ETIK/2018).

**Dosage Determination:** Crude Fucoidan with a dosage variation of 50, 100 and 200 mg/kg body weight were dissolved in a 0.1% Na-CMC suspension and administered orally once daily using the gastric cannula.

**Determination of Bleeding Time:** Mice were inserted into the holder after which its tail was cleaned with 70% alcohol and then cut to a depth of 2 mm using surgical scissors. The blood dripping from the cut was absorbed by filter paper. The time interval between the cut and the first blood drips was calculated as the bleeding time [3, 8, 9].

**Determination of Coagulation Time:** Blood samples were taken through the orbital sinus using a capillary tube which has been broken to 0.5cm long every 15 second until the fibrin thread is formed. The coagulation time is determined between the time when the first blood drips until the fibrin thread is formed for the first time [3, 8, 9].

**Anti-platelet Aggregation Effect Test:** Blood samples were taken through the orbital sinus using a capillary tube. The resulting blood was collected through the use of eppendorf tubes, 3.18% sodium citrate was added and then centrifuged at a speed of 1500 rpm for 15 min. The blood plasma produced was 250 µl after which 0.9% NaCl was added up to 3 ml. However, plasma absorption was carried out using a UV-Vis spectrophotometer at a wavelength of 600 nm. Plasma uptake was measured again after 30 µl of ADP 5 µM was administered as induction of platelet aggregation and incubated for 25 min at 37 °C (3, 9, 13). The percentage inhibition of platelet aggregation was calculated as follows:

Percentage inhibition (%) = [1-(platelet aggregation of sample/platelet aggregation of control] × 100%

**Statistical Analysis:** All values are expressed as mean ± SD of at least triplicate samples. Statistical analysis were assessed using one-way ANOVA. A p-value < 0,05.

**RESULTS AND DISCUSSION:**

**Determination of Bleeding Time:** The bleeding time at 0 day was measured to determine its value before treatment. In normal conditions, it averagely ranges between 50.25 - 57.30 sec. Previous studies reveal that the normal range for mice is between

50.06 - 61.10 sec [9]. Based on the results of statistical analysis, the 0 day value in all treatment groups had no significant difference (p>0.05). The average value for all groups except the normal group after 9 days ranged between 98.63 – 242.00 sec.

**TABLE 1: THE AVERAGE BLEEDING TIME OF ALL TREATMENT GROUPS**

| Treatment group | Average bleeding time (sec) | Average bleeding time (sec) |
|---|---|---|
| | Day 0 | Day 9 |
| Normal Control | 55.31 ± 1.63 | 55.82 ± 1.76 |
| Clopidogrel (Positive Control) | 55.41 ± 1.88 | 242.00 ± 2.35 |
| Crude fucoidan dose of 50 mg | 52.67 ± 1.79 | 98.63 ± 1.80 |
| Crude fucoidan dose of 100 mg | 53.91 ± 1.68 | 151.88 ± 2.28 |
| Crude fucoidan dose of 200 mg | 53.52 ± 1.85 | 209.45 ± 2.17 |

The results showed that positive control and crude fucoidan dose of 50 mg, 100 mg and 200 mg can increase bleeding times and this significantly differ for normal controls. It also revealed that there is a significant difference in the value across all groups after the 9th day (p>0.05).

This study found that there was an increase in bleeding time in the positive control group of clopidogrel at a dose of 75 mg/kg by 242 seconds compared to normal controls (9) while in the crude fucoidan group dosages of 50 mg, 100 mg and 200 mg, increased it by 98.63, 151.88 and 209.45 seconds respectively **Table 1**.

**TABLE 2: THE AVERAGE COAGULATION TIME OF ALL TREATMENT GROUPS**

| Treatment group | Average coagulation time (sec) | Average coagulation time (sec) |
|---|---|---|
| | Day 0 | Day 9 |
| Normal Control | 55.31 ± 1.63 | 55.82 ± 1.76 |
| (Positive Control) | 54.33 ± 3.22 | 55.28 ± 3.03 |
| Crude fucoidan dose of 50 mg | 53.16 ± 3.11 | 152.80 ± 3.87 |
| Crude fucoidan dose of 100 mg | 51.63 ± 2.69 | 78.04 ± 3.07 |
| Crude fucoidan dose of 200 mg | 53.25 ± 3.49 | 99.58 ± 2.44 |

**Coagulation Timing:** Coagulation time was measured at 0 day to determine its value before treatment. In normal conditions, the average value ranges between 54.33 to 55.28 sec. The results of the experiment showed that the average value for all treatment groups except the normal control group ranged between 51.63 – 152.80 seconds on the 9th day. A significant difference (p <0.05) was observed for all the groups after day 9. The value increased for all the groups except the normal group on the 9th day.

**Decrease in Plasma Uptake Between 0 and 9 days:** Decrease in plasma uptake was measured on day 0 to determine its value before treatment. The results showed that the average value in normal conditions ranged between 32.04% - 37.80%. Previous studies reveal that the average value is around 33.22% - 34.91% (22). Therefore, it was discovered that its value on day 0 had no significant difference (p>0.05).

**TABLE 3: AVERAGE REDUCTION IN PLASMA UPTAKE**

| Treatment group | Average reduction in plasma uptake (%) | Average reduction in plasma uptake (%) |
|---|---|---|
| | Day 0 | Day 9 |
| Normal Control | 33.13 ± 1.41 | 32.56 ± 1.15 |
| Positive Control | 32.84 ± 0.78 | 18.00 ± 0.52 |
| Crude fucoidan dose of 50 mg | 34.06 ± 1.07 | 27.31 ± 0.67 |
| Crude fucoidan dose of 100 mg | 35.11 ± 1.79 | 24.61 ± 0.67 |
| Crude fucoidan dose of 200 mg | 34.80 ± 1.69 | 20.26 ± 0.82 |

The average value to range from 18% - 27.31% in all treatment groups except the normal control group. It was also discovered that there is a decrease in absorption after the addition of ADP in the positive control group and the group dose of fucoidan crude. From the results, it can also be seen that the higher the concentration of fucoidan crude, the higher the decrease in plasma uptake. Therefore, a significant difference (p<0.05) was observed for all groups on the 9th day and this was further tested using the Smallest Significant Difference test (LSD).

This research made use of clopidogrel with a concentration of 75 mg/kg BW as the positive group because it belongs to a class of anti-platelet drugs that have the ability of inhibiting platelet aggregation as ADP receptor antagonists, induction of human platelets. Findings showed that the average percent decrease in plasma uptake was 18.00%. The values for positive control groups were compared with those of crude fucoidan at 50 mg, 100 mg, and 200 mg to see whether platelet

anti-aggregation from the study sample had approached the ability of circulating clopidogrel.

The comparison showed that crude fucoidan doses of 50 mg, 100 mg and 200 mg had the value of 27.31%, 24.61% and 20.26% respectively. This shows that 200mg crude fucoidan dose has good platelet anti-aggregation ability.

The decrease in plasma absorption happens because of clopidogrel which causes irreversible inhibition of P2Y12 receptors on platelets so that the ADP (Adenosine Diphosphate) responds to platelet aggregation (6, 7, 10) while fucoidan compounds can prevent thrombocyte induced platelet aggregation. Thrombin can activate platelets through G-protein receptors activated by protease-1 (PAR-1) which causes inhibition. Flavonoids can prevent platelet aggregation by inhibiting cyclo-oxygenase enzyme activity so that it can reduce thromboxane A2 synthesis. It can also suppress various stages of atherosclerosis formation, endothelial damage, leukocyte activation, adhesion, aggregation, and platelet secretion [4, 5, 7].

**CONCLUSION:** It can be concluded that crude fucoidan at a dose of 50 mg, 100 mg and 200 mg showed different results at bleeding time, coagulation time and decreased plasma uptake. The results showed that this treatment group can prolong the bleeding and coagulation time and can also increase the decrease in plasma uptake in mice after the addition of ADP on day 0 and day 9.

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**CONFLICT OF INTEREST:** The authors declare that they have no competing interests

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