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Research Article

Role of Alkaloid on Platelet Aggregation and Serotonin in Migraine

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ABSTRACT

Migraine is a sterile neurogenic inflammation of the trigeminal nerve which releases vasoactive neuropeptides and activates platelets to release vasoactive substances such as serotonin (5-HT). Platelet hyperaggregation occurs in the pathogenesis of migraine caused by one of the stimulatory factors 5-HT. Platelet aggregation is increased and 5-HT levels are elevated in the blood and brain in the early stages of migraine. Alkaloid β-carbolin alkaloids can increase monoamines in brain regions through inhibition of monoamine oxidase (MAO) and inhibition of 5-HT reuptake. Alkaloids in the ethanolic extract of SCE function as analgesics and anti-inflammatory which can reduce pain and improve blood circulation. *Sargassum cristaefolium* extract (SCE) was measured for its bioactive substance content. The extract was administered to an animal model of intraperitoneal nitroglycerin-induced migraine and examined for platelet levels, platelet aggregation and 5-HT. The results of statistical tests showed an increase in platelets ($p<0.05$), an increase in platelet aggregation ($p<0.05$) and a decrease in 5-HT ($p<0.05$). The relationship between alkaloids and platelets; platelets and platelet aggregation; platelet aggregation and 5-HT and 5-HT levels and migraine incidence ($p<0.05$). The alkaloids found in SCE can lower platelet count, decrease platelet aggregation and increase 5-HT levels in migraines.

Key words: Alkaloid, Migraine, Platelet, Platelet aggregation, Serotonin.

INTRODUCTION

Migraine is a disease with complaints of headaches is the most common worldwide, and the cause of the most frequently consulted. Several studies have shown that in migraine there is a sterile inflammatory process in the trigeminal nerve which releases vasoactive neuropeptides, causing arteriolar and arterial vasodilation and activating platelets to release vasoactive substances such as 5-HT.[1] Migraines have decreased plasma 5-HT levels. Serotonin (5-HT) is generally considered to be a pain-inhibiting agent and is a neurotransmitter for the decreased pain inhibitory pathway.[2]

In migraine pathophysiology serotonin (5-HT) is a vasoconstrictor and its plasma level is low. In intracranial vessels, the abundantly expressed 5-HT1B receptor can induce selective vasoconstriction in cranial arteries. In addition to the involvement of vascular 5-HT receptors, in migraine other pathways can also be affected, such as prejunctional 5-HT receptors on trigeminal fibers, central 5-HT receptors and inhibition of CGRP release.[3]

In the pathogenesis of migraine there is platelet hyperaggregation caused by stimulatory factors [eg, collagen, thrombin, adenosine diphosphate (ADP), serotonin (5-HT), thromboxane A2 (TXA2)]. 5-HT is released in excess from platelets. In migraine there is hyperaggregation of platelets. In the early stages of migraine, platelet aggregation increases and causes 5-HT levels to rise in the blood and brain. Therefore, migraine is thought to be preventable by inhibiting platelet aggregation.[4]

Sargassum cristaefolium extract (SCE) contains bioactive flavonoids, alkaloids, and tannins. Flavonoid compounds and tannins are thought to have antioxidant, antitumor, antiviral, anti-inflammatory and antibiotic activities. Alkaloids function as analgesics and anti-inflammatory which can reduce pain and increase blood circulation. The ability to bind free radicals and prevent the formation of free radicals indicates that apart from being an analgesic, antioxidants also have anti-inflammatory functions.[5-7]

MATERIALS AND METHODS

Ethical approval

The Research and Ethics Committee of the Faculty of Veterinary Medicine, Universitas Airlangga, Indonesia has approved this research.

Procedure

This research was conducted on rats induced with nitroglycerin i.p. a dose of 10 mg/kg for migraine. The model rat showed characteristic symptoms such as ear redness, frequent scratching of the head with the forelegs, photophobia followed by hypoactivity and restlessness. The rat model was given SCE via oral nasogastric tube at a dose of 500 mg/kg BW. Four hours after administration of SCE migraine induction, blood samples from all mice were taken. Platelets, platelet aggregation and 5-HT from blood samples were studied through the ELISA kit, following the instructions given by the manufacturer at the Laboratory of the Faculty of Veterinary Medicine, Universitas Airlangga.

Statistical analysis

The results were expressed as mean ± standard deviation, intergroup test and regression test. significance level for statistical analysis $p < 0.05$ was considered statistically significant.

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RESULTS

The ethanol SCE contains flavonoid, alkaloid and tannin bioactive substances. The results of the bioactive phytochemical test of SCE showed flavonoid content of 2.22 QE mg/g extract, alkaloid content 7.71 ASE mg/g extract and tannin content 6.56 mg/g extract (Table 1).

The experimental animal used in this study was Wistar rat *(Rattus novergicus)*. The experimental animals were measured their body weight before and after the experiment. The results of the normality test of the experimental animal body weight data showed a normal distribution, which means that there was no clustered distribution in a certain weight distribution. The results of the homogeneity test showed that the initial animal body weight had a homogeneous variant. The results of the descriptive test of the mean and standard deviation of experimental animal body weight, platelets, platelet aggregation and 5-HT (Table 2). The results of statistical tests showed that there was a significant difference between the platelet count and 5-HT levels between the control group and the migraine group and the group that was given SCE and the group given SCE on platelet aggregation However, the results of statistical tests also showed that there was no significant difference between the control group and initial body weight and final body weight of experimental animals (Table 3).

The results showed that the relationship that had a positive direction was platelet and platelet aggregation; platelet aggregation and 5-HT levels. While the administration of alkaloid and platelets; 5-HT levels and the incidence of migraine have a negative relationship, which means it decreases (Table 4).

DISCUSSION

The use of mice as model mice is used to study human biological factors based on phylogenetic, anatomical, physiological relationships and behavioral similarities with humans. The use of mice is more reliable, because the network linking genes to disease is very likely to differ between species. Other considerations are easy to obtain, inexpensive, easy to care for, easy to survive and adapt to the environment, short life cycle, small size so that it is easy to place and requires little space, easy to breed in the laboratory, has many inbred strains so that it has abundant genetic resources.[8,9]

Intravenous use of nitroglycerin or glyceryl trinitrate causes one of the side effects of moderate-intensity throbbing headache. Repeated administration of nitroglycerin can increase the mean platelet volume, decrease the number of platelets, increase pain transmission, and cause an inflammatory process similar to that of migraine.[10,11]

While migraines have increased platelet aggregation and platelet aggregation, often insufficient aggregation can lead to the release of 5-HT for migraine attacks. The role of platelet aggregation in migraine is demonstrated by the increased activity of platelets during attacks. Migraine attacks with or without aura can be caused by an increase in plasma 5-HT released by platelets, although at different concentrations. At high concentrations, 5-HT can cause vasoconstriction and cause neuronal aura signs, whereas at low concentrations, serotonin can form nitric oxide, prostaglandins and neuropeptides to stimulate perivascular pain fibers and cause vasodilation.[12]

Activation of platelet aggregation can increase intracellular calcium levels in platelets. This can lead to the release of 5-HT and adenosine diphosphate (ADP) from dense platelet granules and ADP granules and 5-HT increases aggregation, and production of thromboxane (thromboxane A2, TXA2) and platelet activating factor. Aggregation is controlled by the formation of adenosine, nitric oxide (NO), the breakdown product of ADP. In migraine, there is a decrease in plasma PGI2 levels. These endogenous platelet antagonists are mainly produced by endothelial COX-2.[4,12]

Involvement of 5-HT in migraine, namely the increase in 5-hydroxyindoleacetic acid (5-HIAA), the main metabolite of serotonin, in urine during migraine attacks. Receptors, enzymes and transporters,

Table 1: Bioactive levels of SCE.

Parameter	Level (mg/g)
Flavonoid	2.22
Alkaloid	7.71
Tannin	6.56

Table 2: Data distribution in all groups.

	Control	Migraine	SCE (mg/kgBB)	p
Initial Weight (g)	175.50 ± 15.61	191.33 ± 10.35	162.50 ± 6.16	< 0.05
Final Weight (g)	200.00 ± 34.98	185.50 ± 9.89	173.50 ± 11.10	0.061
Platelet (10^3/mm^3)	245.00 ±6.87	322.00 ± 6.96	310.17 ± 14.66	<0.05
Platelet aggregation (%)	12.43 ± 7.07	39.39 ± 13.05	17.49 ± 8.44	<0.05
5-HT (ng/mL)	27.570 ± 3.671	17.320±13.483	25.813±10.702	<0.05

Table 3: Test results between groups.

Group	Initial Weight (g)			Final Weight (g)			Platelet (10^3/mm^3)			Platelet Agregation (%)			5-HT (ng/mL)		
	Control	Migraine	SCE	Control	Migraine	SCE	Control	Migraine	SCE	Control	Migraine	SCE	Control	Migraine	SCE
Control		0.685	0.850		0.901	0.504		0.000	0.000		0.000	0.004		0.029	0.038
Migraine	0.685		0.172	0.901		0.948	0.000		0.219	0.000		0.005	0.029		0.001
SCE	0.850	0.172		0.504	0.948		0.000	0.219		0.044	0.005		0.038	0.001	

Table 4: Test regression and signification values.

	Beta (b)	P
Alkaloid - Platelet	-0.292	0.018
Platelet - Platelet aggregation	0.503	0.005
Platelet aggregation - 5HT	0.286	0.026
5HT - migraine	-0.274	0.035

are the main mediators that regulate and maintain serotonin levels in the brain and periphery. Serotonin is distributed in neuronal and non-neuronal tissues such as the cardiovascular, gastrointestinal, kidney and blood systems. Serotonin (5-HT) regulates physiological functions through receptors, most of which are G protein-coupled receptors that activate intracellular second messenger cascades to mediate neural transmission.[13] 5-HT causes smooth muscle contraction and relaxation and migraine symptoms. The central sensitization phase of migraine and pain induction is caused by inflammatory components released from the dura mater, such as potassium ions, protons, serotonin, bradykinin, prostaglandin E2 in cerebral vasculature and nerve fibers that can cause headaches.[14]

Serotonin (5-HT) is a pronociceptive mediator in the periphery and has been shown to be involved in the trigeminal pain process. Different 5-HT receptor subtypes are known to be involved in 5-HT-induced pain processes, namely 5-HT1B, 5-HT1D, 5-HT2A, and 5-HT3A receptors. Triptans are migraine medications that are 5-HT1B/1D receptor agonists. The 5-HT1B/1D receptor is a subtype of serotonergic receptor located in the central nervous system, which functions as a vascular vasoconstrictor. In the pathogenesis of migraine there is also dura mater vasodilation.[2,15]

Serotonin (5-HT) is considered a pain inhibitory agent and is one of the main neurotransmitters of the pain inhibitory pathway. In persistent pain model mice, 5-HT deficiency was associated with decreased thermal hyperalgesia and mechanical allodynia. The 5-HT receptor subtypes have different roles. The 5-HT1 and 5-HT3 receptors are antinociceptive, while the 5-HT2A, 5-HT3 and HT7 receptors are considered pronociceptive. Thus, 5-HT can be both pro and antinociceptive depending on its concentration, affinity and receptor type. It can be concluded that the pathophysiology of migraine is an imbalance in the pain modulation system caused by high interictal brain 5-HT levels and changes in the expression of 5-HT receptor subtypes, resulting in increased pain.[2]

β-Carbolin is part of a group of naturally occurring alkaloids that give rise to a pyridode-3,4-tricyclic ring which has a similar structure to trypamine. β-Carbolin alkaloids can increase monoamines in brain regions through inhibition of monoamine oxidase (MAO) and inhibition of 5-HT reuptake. MAOs are a family of amines containing flavin oxidoreductases, and usually located outside the mitochondrial membrane, also catalyzing the oxidative deamination of monoamines. There are two isoforms of MAO, namely, MAO-A and B. MAO-A metabolizes specifically the 5-HT molecule, while MAO-B binds phenylethylamine and benzylamine.[16]

CONCLUSION

This study proves that the alkaloids contained in SCE can reduce platelet count, decrease platelet aggregation and increase 5-HT levels so that it can reduce the inflammatory process and pain in migraine events.

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CONFLICTS OF INTEREST

The author has no conflicts to declare.

ABBREVIATIONS

ADP: Adenosine Diphosphate; g: Gram; MAO: Monoamine Oxidase; mg: Milligram; ng/ml: Nanogram per mililiter; mm: Milimeter; QE: Quercetin Equivalent; SCE*: Sargassum cristaefolium* extract; TXA2: Thromboxane A2; 5-HT: Serotonin.

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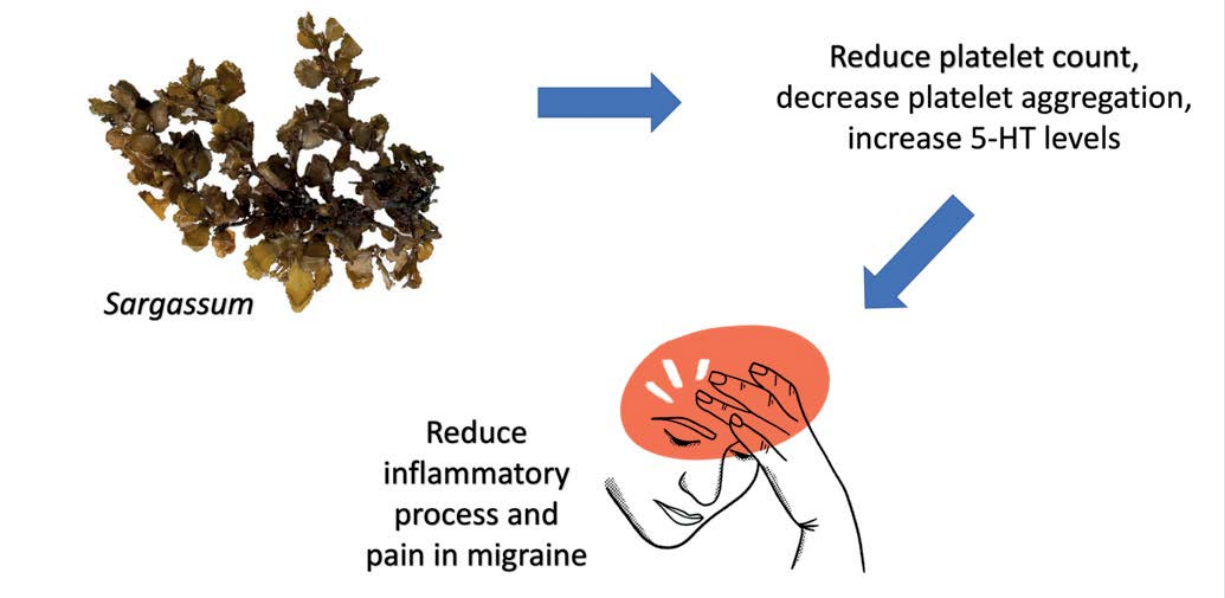
GRAPHICAL ABSTRACT



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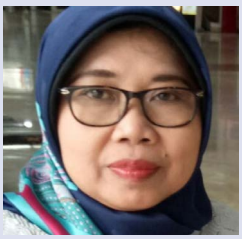

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