ORIGINAL ARTICLE

Antimitotic Activity of Pigeon Pea Filtrates (*Cajanus cajan*) to Sea Urchin (*Diadema antillarum*) Embryonic Cells

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ABSTRACT

Introduction: Cancer is the second leading disease responsible for causing death worldwide. Under normal circumstances, cells of living creatures undergo division. However, excessive cell division is one of hallmarks of cancer. Efforts are used to treat cancer with low side effects by using herbs which are potential to act as anti-mitotic substances, such as pigeon pea (*Cajanus cajan*) which contains flavonoids, tannins, alkaloids, saponins, cyanogenic glycosides, glycosides, and anthocyanins which have the anti-inflammatory, anti-cancer, immunomodulatory, and also antioxidants properties. The study aims to determine the anti-mitotic activity of the pigeon pea (*Cajanus cajan*) filtrates to sea urchin (*Diadema antillarum*) embryonic cells. **Methods:** This study was experimental research in laboratory. This study used 5 replications for each dose of filtrates (5%, 10%, 15%, 20% and 25%) and cell division processes was observed at following specific time: every 15 minutes until the first division stage (to produce 2 cells), every 1 hour for the second division stage (to produce 4, 8, 16, and 32 cells) and every 24 hours to microscopically observe the blastula, gastrula and plateus. The results were observed in 10 high-power fields. Data were then analyzed with an ANOVA test using IBM SPSS 25. **Results:** The result shows that on every treatment groups there was a decrease in the percentage of sea urchin embryonic cells which underwent division at each stage of cell division and was significantly different ($P<0.05$). **Conclusion:** Pigeon pea filtrates showed an antimitotic activity to sea urchin embryonic cells ($P<0.05$).

Keywords: Pigeon pea (*Cajanus cajan*), Antimitotic activity, Sea urchin (*Diadema antillarum*) embryonic cells

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INTRODUCTION

Cancer is the second leading disease responsible for causing death worldwide (1). Based on data from Globocan, the "International Agency for Research on Cancer" (IARC), it was known that cancer caused death of approximately 9.6 million people in 2018 (2). Nationally, in 2018 the prevalence of cancer in the population of all ages in Indonesia was 1.79% or an estimated 1.017.290 people (3).

One of the efforts made by the Indonesian government to control cancer is through preventive approaches such as screening with several methods and also carried out by treatment which acts to stop the growth of malignant or cancer cells such as surgery, radiation and chemotherapy (4). However, cytostatic (chemotherapy) drugs with no side effects on normal cells have not been found to date. Therefore, to reduce or avoid the side effects of conventional medicine, natural ingredients such as treatments with herbal which possess potential as anti-mitotic agents with low side effects are needed to repair damage or impairment of cell division (5).

In Indonesia itself, one of the natural ingredients that can easily be found in house yards and rice fields is pigeon pea. In general, people only use pigeon pea as a vegetable as a complementary staple food to support their food needs (6). The content contained in pigeon pea is also used in traditional Chinese and Brazilian medicine, namely to treat diabetes, dysentery, hepatitis, bedsores, malaria, coughs, and to treat wounds (7). Pigeon pea (*Cajanus cajan*) contains flavonoids (8-10), tannins (8,11), alkaloids, saponins, cyanogenic glycosides, glycosides, and anthocyanins (8,12). The chemical content in pigeon pea (*Cajanus cajan*) has anti-inflammatory, anti-cancer, immunomodulatory, and also antioxidant properties

that play a role in healing inflammation and reducing pain (8,12). Based on the compound content of pigeon pea, and the lack of public knowledge about the use of pigeon pea, especially in the health sector, it is necessary to determine the anti-mitotic activity of the pigeon pea (*Cajanus cajan*) filtrates to sea urchin (*Diadema antillarum*) embryonic cells.

MATERIALS AND METHODS

This research was an experimental study to determine the anti-mitotic activity of pigeon pea (*Cajanus cajan*) filtrates to sea urchin (*Diadema antillarum*) embryonic cells. The object of the research was the pigeon pea filtrates obtained from cleaned, dried, refined, and sieved pigeon pea. A blender was used for the refining process and 500-mesh sieving device was used for sieving. The filtrates were then made into dilution at concentration of 5%, 10%, 15%, 20% and 25% from pigeon pea flour which was formerly prepared. Sea urchins as the experimental animal in this study were collected from Lembar, Pemegatan Beach, West Lombok. Only adult sea urchins with gonads were used.

Research on the anti-mitotic activity of pigeon pea filtrates to sea urchin embryonic cells was carried out by collecting sea urchin eggs and sperm cells. 1 mL of 0.5 M potassium chloride (KCL) was injected through the oral part of sea urchins into the body cavity (intra-coelomic). 1250 µl of egg cells and 250 µl of sperm cells were put into a sterile petri dish containing 25 ml of sterile seawater and incubated until a fertilization membrane was formed. After the fertilization membrane was formed, 1500 µl of pigeon pea filtrates were added (ratio 1: 1) to the treatment group. Furthermore, cell division processes was observed at following specific time: every 15 minutes until the first division stage (to produce 2 cells), every 1 hour for the second division stage (to produce 4, 8, 16, and 32 cells) and every 24 hours to microscopically observe the blastula, gastrula and plateus in the control group and the treatment group under a light microscope in 10 fields of view with an objective magnification of 40x and used 5 replications for each dose of filtrates (13). Data were then analyzed with an ANOVA test using IBM SPSS 25.

RESULTS

The observation results of anti-mitotic activity of pigeon pea filtrates at various concentrations of 5%, 10%, 15%, 20%, 25% to sea urchin embryonic cells can be seen in the Table I.

The observation of antimitotic activity at various concentrations of pigeon pea filtrates showed that there was a decrease in the percentage of sea urchin embryonic cells which underwent division at each concentration. Based on statistical test using ANOVA showed a significant difference between sea urchin embryonic cells that underwent division at each various concentrations (P <0.05).

Table I : Observation Results of Anti-Mitotic Activity of Pigeon Pea Filtrates at various concentrations of 5%,10%, 15%, 20%, 25% to Sea Urchin Embryonic Cells

Treatment	Mean of sea urchin embryonic cells which underwent division (%) ± SD	P
5%	56.22 ± 39.566	
10%	50.67 ± 42.626	
15%	39.56 ± 42.553	.003
20%	34.22 ± 43.005	
25%	28.22 ± 42.337	

The observation results of anti-mitotic activity of pigeon pea filtrates at various stages of cell division to sea urchin embryonic cells can be seen in the Table II.

Table II : Observation Results of Anti-Mitotic Activity of Pigeon Pea Filtrates at various stage of cell division to Sea Urchin Embryonic Cells

Stage of Cell Division	Mean of sea urchin embryonic cells which underwent division (%) ± SD	P
2 cells 1 hour	71.33 ± 24.549	
4 cells 2 hour	62.33 ± 27.869	
8 cells 3 hour	44.33 ± 38.103	.000
16 cells 4 hour	30.00 ± 39.517	
32 cells 6 hour	22.00 ± 40.299	
Blastula 24 Jam	16.67 ± 40.825	

The observation of antimitotic activity at various stages of cell division to sea urchin embryonic cells showed that there was a decrease in the percentage of sea urchin embryonic cells that underwent division at each stage of cell division. Based on statistical test using ANOVA showed a significant difference between sea urchin embryonic cells that underwent division at each stage of cell division (P <0.05).

DISCUSSION

Pigeon pea (*Cajanus cajan*) filtrates at concentrations of 5%, 10%, 15%, 20% and 25% were used in this study to observe the variation of expected division of sea urchin embryonic cells.

The results in this study showed that administration of pigeon pea filtrates at various concentrations of 5%, 10%, 15%, 20% and 25% in each treatment had

the ability to inhibit division of sea urchin embryonic cells with different inhibition results at each stage of cell division and have significantly different result ($P < 0.05$).

These data showed that the administration of higher concentration of pigeon pea filtrates led to a greater anti-mitotic activity of pigeon pea filtrates to sea urchin embryonic cells. This might be because there was higher content of chemical compound in filtrates at higher concentration which can inhibit the division of sea urchin embryonic cells. The effect of pigeon pea filtrates on the embryonic cell was evidenced by the decrease in the percentage of sea urchin embryonic cells that underwent division, in line with the increasing concentration of pigeon pea filtrate given in each treatment and have significantly different result ($p < 0.05$). A decrease in the percentage of sea urchin embryonic cells that underwent division was also seen at each stage of cell division, in line with the length of time observed, the longer the time to observe the cell division stage, the less the percentage of embryonic cells that underwent division and have significantly different result ($p < 0.05$).

Based on the findings of this study, the compounds which are presumed to have anti-mitotic activity to sea urchin embryonic cells are alkaloids, flavonoids, and anthocyanins. This is because those compounds of pigeon pea (*Cajanus cajan*) have the anti-inflammatory, anti-cancer, immunomodulatory, and antioxidant properties which play a role in healing inflammation and reducing pain (8,12). This is also associated with other studies that proved the ability of these compounds to inhibit cell division. Research by Mohan and Jeyachandran (14) showed that alkaloids play a major role as anticancer agents by inhibiting DNA replication, inducing apoptosis and gene expression. Another study regarding the characteristics of daidzein isoflavones of pigeon pea in rat blood showed that the alkaloids in the pigeon pea had a potential as a natural estrogenic (6), in addition to their ability to inhibit division of lung cancer cell (15). A similar research related to the pigeon pea, a study about anticancer activity of pigeon pea (*Cajanus cajan*) leaf extract proved that the effects of flavonoids in the "methanol extract of pigeon pea leaves had anticancer activity against colon cancer cells type WiDr" (16 p.7), while flavonoid was able to inhibit or kill cancer cells in various ways, including as an antioxidant by inactivating oxygen radicals, increasing the rate of apoptosis, inhibiting cell proliferation, and inhibiting DNA oxidation (16). Another research on these compounds was also performed by Sundayani (17) which proved that anthocyanin compounds played an important role in the antimitotic activity of sea urchin embryonic cells.

CONCLUSION

According to the results of this study, it was concluded that the pigeon pea filtrates had an anti-mitotic activity to sea urchin embryonic cells.

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