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The potential of shark bone powder in breast cancer inhibition (pre-clinical study in DMBA-Induced Sprague Dawly Rats)

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Abstract. Breast cancer is a malignant disease, which lead to second cause of that after cervical cancer in women. To date, lots of drugs and supplement have been developed and consumed by patients. Shark bone is one of the supplements that might inhibit the proliferation of cancer cells. The application of shark bone powder for supplementation in breast cancer cases still becomes controversy; but until now people are still many who consume as a supplement. This study aimed to prove the potency of shark bone powder in the inhibition of breast cancer proliferation and to propose the possibility of its biological mechanism. The pre-clinical experimental study used a controlled posttest controlled design with 25 white rats strains of DML-induced Sprague-Dawley strains. The cancer markers observed were p53, AgNORs, VEGF, Bcl-2, and Cas-3. The test subjects were divided into 3 groups: control group and 2 treatment groups fed modified with 60% and 90% respectively. A pre-clinical trial of shark bone powder showed that there was significant inhibition for the DMBA-induced anti proliferation and breast cell cancer ($p < 0.05$) parameters. Optimal concentration of shark bone powder to inhibit breast cancer proliferation lies in concentration 30mg/BB/day.

1. Introduction

Cancer is a malignant disease that mostly affects women, such as breast cancer, cervical cancer and other lymph cancer. Breast cancer is the second common illness suffered by women after cervical cancer. The disease is able to be detected using cell growth biomarker. The AgNORs, p53, Cas-3 compounds, and Bcl-2 genes are cancer markers used to evaluate the inhibition of breast cancer cell growth. The total number of AgNORs in benign and malignant tumors is different, where AgNOR in malignant tumors tends to increase in number, and thus the number of AgNOR spots can be used as a cancer marker. The wild type of p53 protein may play important role in inhibition of cell proliferation via helicase blocking. The native state of wild p53 proteins is unstable, with short lifespan, and they can be detected using immunohistochemical staining [1]. Patient with breast cancer with positive pro-apoptotic Bcl-2s has a better prognosis compared to the breast cancer patient with negative pro-apoptotic Bcl-2s. The apoptotic phenomenon or autolytic cell mediated by Bcl-2, it can be detected and determined by the occurrence of Cas-3 expression.

Several treatment have been performed ie surgery, chemotherapy and radiation but have not had a significant healing effect and possible will be occurred in future In the community the way alternative



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medicine becomes a trend and is believed to reduce pain and heal. Natural ingredients and fermented foods are widely studied and proven as anti-cancer. Tempe is already known to be an anticancer through its ability as an antiproliferation and apoptogenic [2]. Some ingredients have been proven to reduce the proliferation of cancer cells such as syrup leaves, sambiloto (*Andrographis paniculata*), and much more. From Indonesia's marine wealth is suspected that shark bone can be consumed as a supplement for women from malignant disease.

Shark bone uses as an alternative medicine for thousand years ago. Its bone powder has known contain an ability to prevent and cure from malignant disease. The expected research urgency is fish meal as natural biological material can be produced legally and improve image of shark flour in the academic community and abroad. Scientifically obtained findings can be used to prove that shark flour can be used as a therapeutic agent to decrease the number of cancerous mammary cells due to DMBA administration. The target and contribution to science is to prove the effect of bioactive compound in the shark's bone then develop it become scientifically insurable supplement product.

2. Methods

This research design in this study is randomized controlled group posttest only design. The study was conducted in Laboratory of Pathology Anatomy, Faculty of Medicine Universitas Gadjah Mada and Laboratory of Biology, Faculty of Mathematics and Science, Universitas Negeri Semarang. Subject of research was *Rhynchobatus* sp bone collected from Rembang District's fisheries and farmers for several times. The shark's bone was extracted from dead shark and grinded. This research was used *Rhynchobatus* sp that categorized as vulnerable species, so all catching method and sampling was permitted by government regulation (Figure 1).



Figure 1. Shark bone powder after dried and grinded. The bone powder is applicable to be used as health supplement

Fifteen rat (*Rattus norvegicus*) strain Sprague Dawly was used in this research, placed in the 50 x 50 cm cages, got acclimatization for a week and fed using standard rat feed AIN-93 (*ad libitum*). All rats were divided into three groups and treated using shark powder orally. Chemical compound, 7,12-Dimethylbenz[a]anthracene (DMBA) was used to produce carcinoma in rats.

In control group, the rats was administered using placebo or 0 % of shark bone powder, then T1 group was administered using 30 mg/ Kg BB/ day and 60 mg/ Kg BB/ day for T2 or third group. Several biomarkers were analyzed after 30 days treatment. The biomarkers were calculated as dependent variable such as cells expressing of *p53* genes, AgNORs spotting, cells expressing VEGF, Cas-3 production and *Bcl-2* genes.

Caspase-3 is an intracellular cysteine-requiring aspartate protease that exists as a proenzyme, which is activated during the cascade of events associated with apoptosis. Activities of caspases were determined by chromogenic assays using caspase-3 activation kits according to the manufacturer's protocol (Calbiochem, Merck) with PGs in a dose dependent manner [3].

p53 gene expression were observed and counted with repainting imuno-peroksidase AbMop53/DO7. Vascular endothelial growth factor (VEGF) is an endothelial cell-specific mitogen in vitro and an angiogenic inducer in vivo. AgNORs number of spot, measured by silver nitrate staining method, calculated spotting/100 cells.. Cells expressing *Bcl-2* gene was observed and counted with repainting imunoperoksidase AbMoBcl-2/100/D5. Then, Cas-3 staining was observed and counted with

imunoperoxidase, AbMoCas-3/30S1. The data was processed using SPSS 20 for windows, several feature was applied, such as Kolmogorov-Smirnov test to determine the data normality, the normal data continued analyzed using Anova and LSD.

3. Result and Discussion

Induction of DMBA through intragastric pathways leads to breast cancer and can be felt by palpation in the breasts of experimental animals. As long as DMBA induction takes place, no animal attempts are dead. Animals try to lose weight during DMBA induction ($p < 0.05$).

Table 1. Normality test of parameters (p53, AgNORs, VEGF, Bcl-2 and Cas-3) significant at 5% confidence level score of control and treatment groups

Parameter	Dose	Tests of Normality					
		Statistic	df	Sig.	Statistic	df	Sig.
HE	T0	.225	5	.200*	.958	5	.795
	T1	.169	5	.200*	.974	5	.898
	T2	.170	5	.200*	.972	5	.885
p53	T0	.291	5	.191	.905	5	.440
	T1	.372	5	.022	.828	5	.135
	T2	.250	5	.200*	.814	5	.105
AgNOR	T0	.198	5	.200*	.957	5	.787
	T1	.273	5	.200*	.852	5	.201
	T2	.254	5	.200*	.910	5	.468
Bcl2	T0	.254	5	.200*	.803	5	.086
	T1	.273	5	.200*	.852	5	.201
	T2	.287	5	.200*	.914	5	.490
Cas3	T0	.184	5	.200*	.944	5	.692
	T1	.258	5	.200*	.925	5	.563
	T2	.231	5	.200*	.943	5	.685
VEGF	T0	.216	5	.200*	.933	5	.619
	T1	.242	5	.200*	.893	5	.370
	T2	.256	5	.200*	.843	5	.174

Table 2 Anova of cells expressing p53, spotting AgNORs, Cas-3, Bcl-2 and VEGF cells in breast cancer

Group	HE	p53	AgNORs	Bcl-2	Cas-3	VEGF
T0	81.2 ^a	54 ^a	84.6 ^a	66.8 ^a	22 ^a	84.8 ^a
T1	63.5 ^b	43.8 ^b	79.8 ^a	28.6 ^b	37 ^b	72.4 ^b
T2	49.8 ^c	40.4 ^c	66.8 ^b	30.2 ^c	27.6 ^c	38 ^c

Shark bone is consist of protein (about 40%), glycosaminoglycans (5% -20%), and calcium. Shark cartilage crude extracts supplementation effectives to fight against cancer cells [4]. It shows that on each cancer marker parameters (p53, AgNORs, VEGF, Bcl-2 and Cas-3) have significantly different at 5% confidence level.

Shark bone can suppress the growth of cancer cells, exerts its effects through multiple targets on angiogenesis [5]. It shown by p53, AgNORs and VEGF; in which a third complex of cancer cancers command on suppressing or inhibiting cell proliferation, primarily because there is inhibition of the formation of new blood vessels. This is consistent with the fact that shark bone powders trials can be efficacy in cancer treatment [6,7].

The administration of shark bone powder in this study may decrease VEGF expression, followed by cellular improvements ie decrease AgNORs and p53 but decrease in VEGF in studies has not been in line with the apoptotic strength of cancer cells (Tables 1 and 2). Cancer-supporting tissues can produce VEGF, because of chemotactic signals originating from cancer cells. Giving certain drugs or substances through oral will experience the process of absorption, distribution, metabolism and excretion [8]. The tyrosine kinase Flt-1 (VEGFR-1) and Flk-1 / KDR (VEGFR-2) are high-affinity VEGF receptors. VEGF plays an important role in development of angiogenesis and also important for cell fission.

Substantial evidence also involves VEGF as a pathological angiogenesis mediator. Anti-VEGF monoclonal antibodies and other VEGF inhibitors are able to inhibit the growth of some mouse tumor cells. VEGF markers are also used in clinical trials in various cases of malignancy.

Table 2, shows that VEGF-expressing cells significantly decrease with increasing concentration of shark bone powder. This is followed by a decrease in proliferation of cancer cells, AgNORs and enhancement apoptotic cells in rat cancer cells ei. Bcl-2 and Cas-3 cells, this proves the above statement [9].

4. Conclusion

The optimum doses of shark bone powder, 30 mg/ kg/ day can be used as an inhibition agent in cancer cell proliferative. Similar studies need to be done to strengthen shark bone powder, proven to be a cure for cancer patients.

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