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The Administration of Oralwhite Tea (*Camellia sinensis*) Extract Reduced Tumor Diameter Size of Breast Carcinoma and Head/Necksquamous Cell Skin Carcinoma in Female Sprague Dawley Strain Rat (*Rattus norvegicus)* Induced by 7,12 Dimethylbenz-(α)Anthracene (DMBA)

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Abstract: ***Background:Carcinoma is a disease with high mortality rate. In women, the mostcommon type of carcinoma is breast carcinoma. This carcinoma is associated withhormonal problems, genetic factors and environmental influences. Head/neck skincarcinoma with the most common carcinoma is squamous cell carcinoma, which isa non-melanoma skin malignant tumor originating from the suprabasal epidermalkeratinocytes. Causes of skin squamous cell carcinoma include ultraviolet radiationand exposure to chemicals. White tea (Camellia sinensis) is tea that comes fromvery young tea leaves that are still rolled up when picked and protected from thesun thereby contains the highest levels of polyphenols and caffeine. One of thefunctions of polyphenols is antioxidants and can trigger apoptosis in carcinomacells. This study was conducted to prove that administration of white tea (Camelliasinensis) extract orally reduced the tumor diameter size of breast and head/neckskin carcinoma of female Sprague Dawley strain rats (Rattus norvegicus) inducedby DMBA. Method:This study was conducted using a randomized pretest-posttest only controlgroup design, using 10 female Sprague Dawley rats as research subjects. Subjectswhich were positive for tumors after being induced with DMBA were divided intotwo groups (control and treatment). The control group received a placebo in theform of 0.5 ml aquabidest while the treatment group received 100 mg/kg bodyweight of white tea extract. The extract and placebo were given orally once a dayfor 21 days. Measurement of carcinoma tumor diameter was carried out before andafter treatment. The results obtained are processed using software. Result: The comparative analysis showed that the mean tumor diameter in the control group was 9.80 mm (± 4.15 mm), and in the treatment group was 7.60 mm (± 2.88 mm). There is a difference of 2.20 mm (3.01 mm to 7.41 mm). This difference was not significant with a p value = 0.359. After treatment, the results of the comparative analysis showed that the mean tumor diameter in the control group was 19.00 mm (± 3.08 mm) while in the treatment group was 14.60 mm (± 2.30 mm). These results indicate that there is a difference of 4.40 mm (1.43 to 8.37 mm). This difference is significant with a p value = 0.034. Conclusion:It can be concluded that administration of white tea (Camelliasinensis) extract orally reduced the tumor diameter size of breast and head/neck skincarcinoma of female Sprague Dawley strain rat (Rattus norvegicus) induced byDMBA.***

Keywords: white tea, *Camellia sinensis*, breast carcinomas, head and neck skin carcinomas, diameter size of carcinomas, DMBA

1. Introduction

Breast carcinoma is a malignancy in breast tissue that can originate from the epithelium of the ducts or lobules.[1] Squamous cell carcinoma is the second highest skin carcinoma after basal cell carcinoma. Squamous cell carcinoma is a non-melanoma skin malignant tumor originating from the keratinocytes of the epidermis. The carcinomas are associated with hormonal problems, genetic factors, and environmental influences such as chemical and UV-B exposure.[2,3]

In Indonesia, most of carcinoma cases are found at an advanced stage, where treatment is difficult.[1] Chemotherapy and radiotherapy as a traditional carcinoma treatment not only cured the malignant cell but also killed the healthy cell as well thus given a negative effect to the patient.[4]

Aging and carcinoma are share a quite similar process. In the aging process, changes in the extracellular matrix in malignant tissue are associated with loss of hemostasis of the extracellular matrix of healthy cells and increased stiffness of the body organs.Meanwhile in primary tumors, carcinoma cells form a matrix around them to form substances needed by carcinoma cells. The carcinoma cells then hijack the dynamic reciprocity of healthy cells so that carcinoma cells can grow and harm healthy organs. As a result of stiffness and decreased extracellular matrix, it causes proliferation, ability to continue to grow, invasion and metastasis of carcinoma cells.[5]

Anti-Aging Medicine is a new concept to prevent aging as well as carcinoma. The prevention strategies focus on healthy lifestyle, such as getting an enough rest, being active, eating a healthy food and avoiding such a carcinogenic exposure.A healthy food usually contain a lot of antioxidant.[5,6]

White tea is one of the types of tea obtainedfrom the tea shoots in the form of buds. It doesnot go through

fermentation process; thus, its catechincontentishigher than other type of tea.Catechin, one of polyphenol derivates was found about13.5-31% inwhite tea leaves. Catechin found in tea is a complex compound consists of epicatechin, epicatechingallate, epigalocatechin, epigallocatechin gallate, and gallocatechin. Epigallocatechingallate, in tea can induce apoptosis in carcinoma cells.[7,8,9]

Aging is the decline in physiological organ function over time and is a major risk factor for the development of carcinoma.[6,7]If the aging process can be prevented by the antioxidant, the quality of life will improve, and based on that,we are interested to studywhite tea leaves extract effect on breast carcinoma and head/neck skin carcinoma through reduction in tumor size diameterafter DMBA induction.

2. Method

Study design and sample
This study was conducted using a randomized pretest-posttest only control group design and used 10female Sprague Dawley rats as research subjects. Subjects were healthy rats aged 4 weeks old and weight 130 – 200 grams. Subjects were randomly divided into two groups, control and treatment groups(n=5).

DMBA Induction
A 4 mg/ml DMBA solution induction with a total dose of 17,5 mg/body weight was given orallyto subjects one time each two weeks for six weeks.Furthermore, the induced rats were kept for eight months to determine tumor development.

Measurement of carcinoma tumor
All induced rats were palpatedfrom the head to the base of the tail one week after each induction to assess tumor location and diameter. The rats were given a code according to the location of the tumor, P for tumors in the breast and L for tumors in the head/neck. P1 is the code given to first rat that known having breast tumor, followed by P2, P3, etc. for the next rats. L1 is the code given to first rat that known having head/neck tumor, followed by L2, L3, etc. for the next rats. The PL code was given to rats that has first tumor found on the breast followed by additional tumor on the head/ neck. The LP code was given to rats with first tumors found on the head/neck, followed by breast tumors afterwards. If there is more than one tumor found in rats, tumor with the largest diameter will be measured.

Preparation of tea extract
The tea extract was prepared in Food Technology Laboratory Faculty of AgricultureUdayana University. A refined white tea leaves weighed as much as 400 grams added with 96% ethanol, stirrer for one hour and then soaked for 24 hours. The soaked tea product then filtered in four different extract for each four consecutive days.Then the whole filtrate is concentrated using a rotary evaporator up to one-third of the original volume.

Subject intervention
The control received 0,5 ml distilled water.Treatment group received 100 mg/kg body weight of white tea extract in the form of diluted solution of50 mg/ml distilled water. After 21 days, all rats were anesthetized, and then carcinomas tissue were obtained for histopatological examination. The histopathological preparations were made by performing fixation to tumor tissue using 10% NBF solution. The tissue was cut, arranged in tissue cassettes, dehydrated automatically with a dehydrator, dried in a vacuum machine, and blocked with paraffin liquid. The tissue was then cut into 4-5 µm with a microtome machine, and this piece was attached to the microscope slide. The microscope slide was then stained manually with H&E stain.

Statistical analyses
The results were expressed as means ± SD. The normality of the variables was confirmed by the Shapiro–Wilk test and homogeneity of variance by the Levene'stest. Statistical differences among the groups of treatments were assessed by Independent t-test analyses. The significance level was 95% in all cases ($P < 0.05$). All data were analysed by the computer application

SPSS for Windows (version 17.0, SPSS Inc., Chicago, USA).

3. Result

Table 1 showed the characteristic of tumor diameter in control and treatment subject.

The data between treatment and control group were analyzed using an independent sample t-test and the mean tumor diameter differences between groups are shown in **Figure 1.**

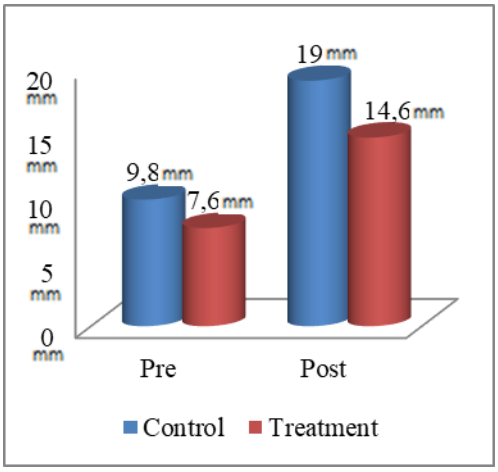

Figure 2: Comparison of the Tumor Size Diameter between Control and Treatment Groups (p=0,034)

There was no significant different in tumor diameter between two groups before intervention, account for 9.80 mm (± 4.15 mm) and 7.60 mm (± 2.88 mm) for control and treatment group, respectively. After intervention, the mean tumor diameter was significantly larger in the control group 19.00 mm (± 3.08 mm)than the treatment group14.60 mm (± 2.30 mm). These results indicate that there is a difference of 4.40 mm (1.43 to 8.37 mm). This difference is significant with a p value = 0.034.

The histology of carcinoma are shown in **Figure 2 and 3.**

Table 1: Descriptive Analysis of Tumor Diameter

Variables	Group	n	Mean ± SD
Pretest Tumor	Control	5	9.80 ± 4.15
	Treatment	5	7.60 ± 2.88
Posttest Tumor	Control	5	19.00 ± 3.08
	Treatment	5	14.60 ± 2.30
Change in Tumor Size	Control	5	9.20 ± 1.30
	Treatment	5	7.00 ± 1.58

*SD : standard deviation

4. Discussion

Carcinoma is a disease with a high mortality rate and is often found in an advanced stage, making its treatment more difficult.Breast carcinoma and skin squamous cell carcinoma caused by hormonal factor, ultraviolet irradiation, and exposure to chemicals.[1-3,10]

In this study, DMBA works as breast carcinoma and head/neck squamous skin carcinoma inducer in female Sprague Dawley rats.[11]The 7,12-dimethylbenz (α) anthracene (DMBA) is a chemical substance in the polycyclic aromatic hydrocarbon (PAH) which is known to be mutagenic, teratogenic, carcinogenic, cytotoxic, and immunosuppressive.[12]One of the metabolic results of DMBA by CYP1 is the formation of ROS cation metabolites, as a source of pro-oxidant reactions. These changes will cause gene mutations that can initiate carcinoma cells.[13]

Teas are naturally producing useful secondary metabolites such as polyphenols and catechins.[7-9]These compounds known as antioxidants to scavenge free radicals in the body and can prevent the development of carcinoma cells in the body.

White tea in particular has known having the highest polyphenols and catechins level among the other type of tea.[14] White tea has very high levels of polyphenols because the tea leaf still rolled up and protected from the sun when picked. Moreover, the tea budsare only dried and do not undergo a fermentation process.[7-9]

It was found that the mean tumor size diameter in the control group was significantly higher than the treatment group (19.00 mm (±3.08 mm) vs 14.60 mm (± 2.30 mm), respectively p = 0,034). This shows white tea leaves extract at a dose of 100 mg/kg body weight role in reducing the adverse effect of ROS exposure compared to the control group who received placebo.

The anti-tumor properties of tea polyphenols are to inhibit the growth of carcinomas, induce apoptosis and cell cycle arrest, inhibit cell invasion and metastasis, and suppress angiogenesis.The mechanism of anti-carcinogenic activity of the catechins in white tea, including suppressing the increase in ROS levels due to carcinogenic induction and protecting DNA damage. Catechins also reduce thedegree of damage due to ROS properties by increasing the antioxidant activity as incatalase enzymes, superoxidase dismutase, glutathione peroxidase which directly destroy ROS.[14,15]

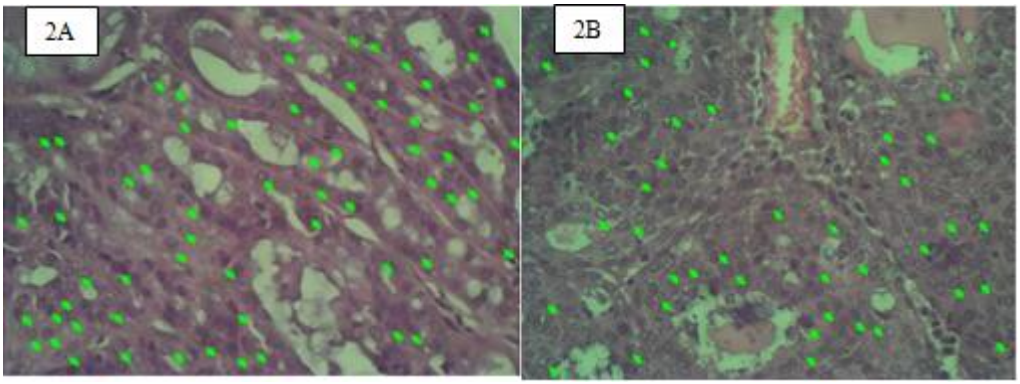

Figure 2. Histopathological features of rats with Invasive Ductal Carcinoma. Abnormal breast cells with hyperchromatic nucleus with irregular edge (green arrow). **Figure 2A.** 100x magnifications and **Figure 2B.** 400x magnifications.

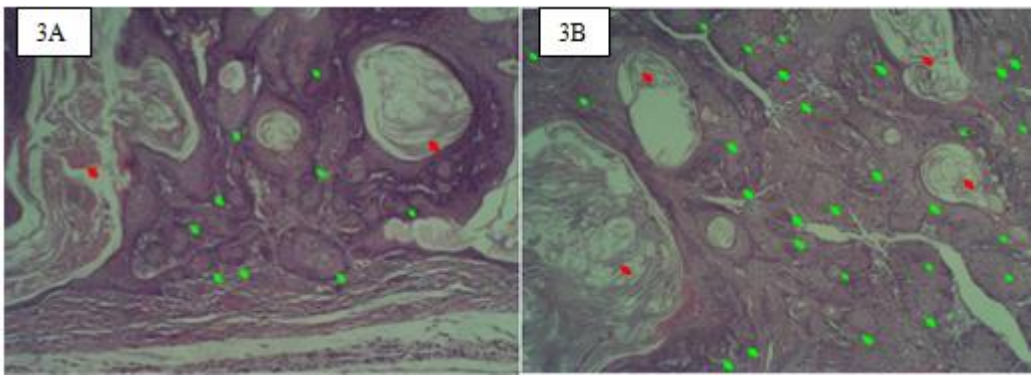

Figure 3: Histopathological features of rats with Squamous Cell Carcinoma. Differentiated squamous cells (green arrow) and keratin pearl stucture (red arrow). **Figure 3A.** 100x magnifications and **Figure 3B.** 400x magnifications.

5. Conclusion

This study proved that oral administration of white tea (*Camellia sinensis*) extract can reduce the tumor diameter size of breast carcinoma and head/neck skin carcinoma in female Sprague Dawley strain rat (*Rattus norvegicus)* induced by 7,12 dimethylbenz-(α)anthracene(DMBA).

6. Ethical Approval

This study had been ethically approved by the ethical commission of the Faculty of Medicine Udayana University with approval letter number 3573/UN14.2.9/PD/2019.

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