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EFFECTS RED DRAGON FRUIT *(Hylocereus Polyrhizus)* PEEL EXTRACT TOWARDS MALONDIALDEHYDE LEVEL IN *Rattus Norvegicus Strain Wistar* EXPOSURED BY CIGARETTE SMOKE

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Abstract: **The peel of red dragon fruit (Hylocereus Polyrhizus) is known have content Flavonoid. Flavonoids in the red dragon fruit peel has the potential to be used as a natural antioxidants. This study aims to analyze the effect of red dragon fruit peel of Malondialdehyde (MDA) plasma level in wistar rats which were exposed to cigarette smoke. Thirty male rats, aged 2-3 months, weight ± 200g. Divided into 5 groups, K- (Normal), K + (Cigarette Smoke), P1 (Cigarette Smoke and Red dragon fruit peel extract 2g /cc), P2 (Cigarette Smoke and red dragon fruit peel extract 4 g /cc), and P3 (Cigarette Smoke and red dragon fruit peel extract 8 g /cc). Provision of red dragon fruit peel extract and exposure to cigarette smoke carried out for 14 days. The blood was taken on the 15th day and the levels of Malondialdehyde measurement done by the TBARS method. Doses of 2 g /cc, 4 g /cc, and 8 g /cc ethanol extract of red dragon fruit peel can lower MDA levels in wistar rat plasma that exposured cigarette smoke (p <0.05). The mean value of plasma MDA level of group K- is 0.237 , K+ is 0.317, P1 is 0.294 ,P2 is 0.275, dan P3 is 0.26.**

Keywords: **Flavonoid, Red Dragon Fruit Peel, Malondialdehyde, Cigarette.**

I. INTRODUCTION

Smoking has become a life style in community, the number of young smokers increases every year with also increased the prevalence of non-communicable diseases. (1) Studies epidemiology about the effect of smoking had been done and proven that smoking may increase the risk of COPD (*Chronic Obstructive Pulmonary Disease*), Pulmonary cancer, and cardiovascular diseases. (2) Moreover, smoking may also increase the likelihood of other diseases in people with HIV / AIDS, including pneumonia and dementia due to AIDS. Smoking also accelerate subclinical TB to become clinical TB, with a higher likelihood of death (3)

The smoking habit was hard to modify because addictive component that contain in the cigarette (3). Cigarettes smoke contains many toxic, mutagenic chemicals, and carcinogenic, as well as stable and unstable free radicals and reactive oxygen species (ROS) with the potensial for biological damage. Cigarettes smoke could be divided into the mainstream (smoke inhaled by smoker) and sidestream smoke. The mainstream is devided into a particulate solid phase (tar) and the gas phase (toxic gases and free radicals). The sidestream smoke is devided into the solid and gas phases with reactive and short lived free radical, toxic and carcinogenic compound. Tar , the solid phase in mainstream, contains remarkably high concentrations of free radicals. Tar can produce superoxide anion (O^{2-}) and subsequently H_2O_2 and the reactive hydroxyl radical ($HO^{\cdot}$), which induces oxidative stress and cause biological damages to cellular membrane lipid, enzymes, proteins and DNA. (4) Oxidative stress is a condition that present of free radical and antioxidants in body are imbalance. (5) Oxidative stress can be indicated by high concentration of Malondialdehyde (MDA) levels in the body(6)

Antioxidants can reduce the occurrence of acute oxidative damage or ROS through electron donor mechanism and end up with lower concentration of MDA level in body. (7) Antioxidant can devided into synthetic and natural. Natural antioxidant could be produced from various parts of plants. Peel, wasted part of the fruit, could be as a natural antioxidant that prevent the oxidative stress.

Red dragon fruit peel could be used as antioxidant, because flavonoids, betasianins, phenolic that contained in the peel . (8). Flavonoid has been shown antioxidant effects and capture of free radicals. (9) Red dragon fruit has better antioxidant power than the other species of dragon fruit. (10)

II. MATERIAL AND METHODE

A. Animals and experimental procedure

This study is an experimental laboratories with a research design that is Randomized Posttest Control Group Design. Our study was approved by Research Ethical Committee of Udayana University, Bali, Indonesia.

Thirty Male white wistar rats (Rattus Norvegicus), around 2.5-3 months old, weighing 150-200 grams, and in good health were assigned randomly into five grups, each group consisting of six rats. The K- group (negative control group), rats that not exposed to cigarette smoke, were not given red dragon fruit peel extract (Hylocereus polyrhizus) and were given standard food. The K+ group (positive control group), the rats exposed to the cigarette smoke, were not given red dragon fruit peel extract (Hylocereus polyrhizus) and were only given standard food. Group P1 (treatment group), rats exposed to cigarette smoke, were given red dragon fruit peel extract (Hylocereus polyrhizus) 2 g/cc,and were given standard food. Group P2 (treatment group), the rats exposed to cigarette smoke, were given the red dragon fruit peel extract (Hylocereus polyrhizus) 4 g/cc, and were given standard food. Group P3 (treatment group), the rats exposed to cigarette smoke, were given red dragon fruit peel extract (Hylocereus polyrhizus) 8 g/cc,and were given standard food.

All groups are acclimatized for seven days and proven standard food and tap water drink ad libitum. On eighth day groups K+, P1, P2, and P3 are exposed to three non-filtered cigarettes smoke in the morning, and in the afternoon the groups P1, P2, and P3 were given red dragon fruit peel extract according to their dosage until twenty-first day.

On the twenty-second day, all the rats blood were taken from intraocular using capillary tube and then feel on ETDA tube for 1cc and they were under anesthesia Then blood were test for MDA using TBARS method.

B. Statistical Analysis

Data were analyzed using the SPSS software program for Windows, version 24.0 (SPSS Inc., Chicago, IL). The results were presented as mean±SD. The normality of the data distribution was confirmed using the Shapiro-Wilk test. The homogeneity of the data was confirmed using levene's test. Then data were analyzed by one-way ANOVA followed by the Least Significant Difference post hoc test. Results were considered statistically significant at $p \leq 0.05$

III. RESULT AND DISCUSSION

A. Result

The results of the measurement of MDA levels of wistar rats given red fruit peel extract after being exposed to cigarette smoke every day for 14 days are presented in Table 1.

Table 1: Mean Malondialdehyde

Group	Mean MDA±SB
K (-)	0.237±0.009
K(+)	0.317±0.012
P1	0.294±0.015
P2	0.275±0.014
P3	0.260±0.015

MDA level in plasma of rats exposed to cigarette smoke only (K+) increased significantly compared to group without cigarette smoke (K-) $p = 0.001$ *($p<0.05$)*. MDA level group K+ decressed siginificantly compered to group P1,P2, and P3. *($p<0.05$)*, but the difference between P2 with P3 was not significant $p=0.072$ ($P>0.05$) in Table 2.

Table 2: Least Significant Difference test of Malondialdehyde level

Group	Mean Difference (95% CI)	P
K- vs K+	0.080	0.001
K- vs P1	0.056	0.001
K- vs P2	0.037	0.001
K- vs P3	0.023	0.007
K+ vs P1	0.023	0.005
K+ vs P2	0.043	0.000
K+ vs P3	0.057	0.000
P1 vs P2	0.019	0.020
P1 vs P3	0.034	0.000
P2 vs P3	0.014	0.072*

Description: (CI= Confident Interval, p= p value) * = No significantly different at p > 0.05

B. Discussion

MDA level between group K- and K+ have increase significantly with mean different 0.08. (Table 1) This is due to cigarette smoke contained various kinds of free radicals that are mutagenic and carcinogenic. These free radicals cause oxidative stress through the mechanism of membrane lipid destruction from cells with one of the end result, malondialdehyde that can detected in the blood.(4,6)

The mean MDA level of the group exposed to cigarette smoke and red dragon fruit peel extract (P1, P2, P3) compared to the group that was only exposed to cigarette smoke (K+) had a significant difference with p <0.05. (Table 1) This is because of the red dragon fruit peel contains antioxidant compounds such as flavonoids, phenolic, betasianin and also contains other substances such as vitamin B1, vitamin B2, vitamin B3, and vitamin C, protein, fat, carbohydrates, carotene, phosphorus, and iron . (11) Inside red dragon fruit contains some antioxidants such as betasianin, phenol and flavonoids, these antioxidants can reduce the amount of free radicals in the body, such as those derived from cigarette smoke, resulting in a decrease in MDA levels. It is known that MDA is one of the biomarkers of oxidative damage in cell membranes that can be detected in blood, as well as one of the results of lipid peroxidation caused by free radicals (12).

Flavonoids are exogenous antioxidants which are useful in preventing oxidative stress. Flavonoids can inhibit enzymes responsible for producing superoxide anion radicals, end of the reaction and extinguish superoxide anion radicals (13)

The activity of In vitro, flavonoid compounds can prevent oxidative stress caused by free radicals by several mechanisms, such as ability to activatie antioxidant enzymes, ability to inhibit oxidases, and Direct scavenging of reactive oxygen species (ROS). Ability to activatie antioxidant enzymes by induce enzymes such as NAD (P) H- quinone oxidoreductase, which is the initial defense enzyme against oxidative stress. ability to inhibit oxidases enzymes, such as xanthine oxidase and protein kinase, which the results were in a decrease in the level of oxidative stresses. Direct scavenging of ROS by donating hydrogen atoms to free radicals so that antioxidants stabilize. (14)

Phenolic compounds are terminators of free radicals and as binders of active redox metal ions. This metal ion allows its role to catalyze lipid peroxidation reactions. These phenolic antioxidants block the oxidation of lipids and other molecules by donating hydrogen atoms to radical compounds to form phenoxyl radical intermediates. The phenoxyl radical intermediate compound is relatively stable so it is no longer able to initiate further radical reactions. (12)

The mean MDA levels of P1 vs P2, and P1 vs P3 were significantly different (P <0.05) but for groups P2 and P3 there were no significant differences (P> 0.05) (Table 2). This shows an increase in the dose of 8 g/cc of ethanol extract of red dragon fruit peel does not provide a significant difference in the decrease in MDA levels compared to 4g/cc. Addition of doses of 4g/cc to 8g/cc can cause antioxidant compounds to be not optimal in stabilizing free radicals and it is likely that compounds become prooxidantants.

Prooxidantant is a characteristic of compounds that can encourage oxidation in cell components involving free radical compounds and lead to chain reactions whereas antioxidants are compounds that can protect cells from the harmful effects of reactive oxygen free radicals. At high concentrations, the antioxidant activity of the phenolic group often disappear even these antioxidants become prooxidant. Antioxidant and prooxidant properties depend on antioxidant structure, conditions and samples to be tested. In this case the structure of the oxidant that was OH group on the base frame. More and more OH becomes both, an antioxidant and a prooxidantant (15)

Besides being an antioxidant, flavonoids can be a prooxidant. The mechanism in vitro of flavonoids as prooxidant are direct prooxidant function of flavonoids and oxidation by flavonoid phenoxyl radicals. The direct prooxidant function of flavonoids is is influenced by the total number of hydroxyl groups in flavonoid molecules, on the study of several studies increasing the dose of flavonoids, in this case an increase in the dose, increased the number of flavonoid molecules, can increase oxidative stress. Oxidation by flavonoid phenoxyl radicals, the result of the direct scavenging of ROS by flavonoids is a phenocyte flavonoid radical that is highly reactive and can undergo further oxidation. (14)

IV. CONCLUSION

Cigarettes are a source of free radicals and red dragon fruit peel is a source of antioxidants. Free radical would increase MDA level, but Antioksidan can inhibit the increasing of MDA level. This is proven by the administration of red dragon fruit peel ethanol extract in rats exposed to cigarette smoke at doses of 2 g /cc, 4 g /cc, and 8 g /cc had lower plasma MDA levels compared to groups exposed to cigarette smoke and not given extracts, with the most significant doses are 2g/cc and 4g/cc. Further studies are needed to pre and post study, effective doses, and mechanism in red dragon fruit peel as antioksidants. .

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