

# Effect of Giving Ethanol Extract of Red Dragon Fruit (*Hylocereus polyrhizus*) on Immunohistochemical Overview of CD40 in Cardiac Aorta of Male Wistar Rat (*Rattus norvegicus*) Induced by Cooking Oil

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## ABSTRACT

Atherosclerosis is a cardiovascular disease that can cause death such as coronary heart disease. The use of used cooking oil can cause the formation of free fatty acids, trans fatty acids and free radicals that can trigger atherosclerosis. One of the biomarkers of atherosclerosis is CD40. Red dragon fruit contains anthocyanins which act as antioxidants and anti-inflammatory. The purpose of this study was to determine the effect of the ethanolic extract of red dragon fruit (*Hylocereus polyrhizus*) (EEBNM) on CD40 immunohistochemistry in the heart aorta of male wistar rats (*Rattus norvegicus*) induced by cooking oil. This research is true experimental with randomized post-test-only control group design. The number of samples was 35 male wistar rats consisting of 7 groups. Group K0 (Standard normal diet). In group P1, P2 was given cooking oil 5 times for frying with EEBNM doses of 60 and 90 mg/kg BW per day. P3, P4 were given cooking oil 10 times for frying with EEBNM doses of 60 and 90 mg/kg BW per day. P5, P6 were given cooking oil 15 times for frying with EEBNM doses of 60 and 90 mg/kg BW per day for 60 days. The results of the Kruskal Wallis test showed that there was a significant difference between the seven treatment groups ( $p$ -value=0.039). The results of the Mann Whitney test in the treatment (P2) of frying 5 times at a dose of 90 mg/kg BW on the CD40 count the results showed a significant difference. This study proves that the ethanolic extract of red dragon fruit (*Hylocereus polyrhizus*) can reduce

the number of CD40 in the heart aorta of male wistar rats induced by cooking oil.

**Keywords:** Atherosclerosis, Cooking Oil, Red Dragon Fruit, CD40

## INTRODUCTION

Cardiovascular disease is a disease that causes many deaths. One of them is coronary heart disease caused by atherosclerosis. In some developing countries such as Africa, India and Southeast Asia, the mortality and morbidity of heart disease due to atherosclerosis are shown. Indonesia has also experienced the same epidemiological transition since the last decade in degenerative diseases due to lifestyle changes that imitate western society and unhealthy and high cholesterol diets (Robins, 2015).

In Indonesia, currently the death rate from coronary heart disease is ranked 3rd from the cause of death. In one year there are 500,000 new cases and 125,000 people die of atherosclerosis (Wijaya, 2011). The prevalence of hyperlipidemia increases from year to year. In 2008 it was recorded at 35.1% then in 2013 it increased to 35.9% (World Health Organization, 2013). The higher the prevalence of hyperlipidemia, the higher the incidence of atherosclerosis which has an impact on death (Nindrea, 2015).

Atherosclerosis is one of the main causes of death involving the inflammatory process of blood vessel walls by hyperlipidemia. The process of atherosclerosis begins with damage or injury to the endothelial layer which is followed by an increase in endothelial cell permeability, increased release of adhesion molecules on the surface of endothelial cells and the production of inflammatory cytokines. Monocytes and T lymphocytes will migrate through the damaged endothelial layer into the intima which will then differentiate into macrophage cells.

CD40/CD40L interaction is an important pathway in the inflammatory response with the production of inflammatory cytokines and is found in arterial plaque from macrophages, smooth muscle cells and T cells which significantly influences endothelial cell function associated with the development of atherosclerosis (Wierzbicki, 2007). CD40 is a receptor and CD40L is a CD40 ligand present on endothelial cells, macrophages.

The consumption of cooking oil per capita of the Indonesian population in 2011 was 8.24 liters/capita/year. In food processing, cooking oil functions as an introduction to heat, flavoring, changes in food texture and an increase in nutritional value. The use of cooking oil for daily cooking is inherent in the community because fried food has a more delicious and savory taste than boiled, steamed or baked food. This makes some people, especially housewives and food vendors, very dependent on cooking oil.

Based on previous research, it is known that 90% of Indonesian people use repeated cooking oil, which is 4-8 times per day. Cooking oil that is repeatedly oxidized (reacts with air) can increase the level of peroxide value (Siswanto, 2015). Heating cooking oil at high temperatures and used repeatedly will damage the oil due to oxidation which can produce aldehydes, ketones, and aromatic compounds that have a rancid odor.

Research conducted on culinary traders who use used cooking oil in Karang Malang Culinary Park shows that the average number of visitors is 80-100 people, but the use of cooking oil to process food is not proportional to the number of visitors. Based on the results of interviews conducted by researchers, it is known that traders only replace cooking oil 2 times where processing cooking oil can be up to 80 times a day; this means that cooking oil is used 40 times for processing fried foods and served to consumers (Aini, 2012).

Based on the above background, the researcher is interested in conducting research on the effect of giving ethanol extract of red dragon fruit (*Hylocereus polyrhizus*) at a dose of 60mg/kg BW and 90mg/kg BW on the immunohistochemical features of CD40 in the heart aorta of male Wistar rats (*Rattus norvegicus*) induced by cooking oil. 5 times, 10 times, and 15 times the frying pan.

## RESEARCH METHODS

This type of research is true experimental with randomized post-test only control group design and followed a completely randomized design (CRD), using a control group and a treatment group, each of which was given different treatment. True experimental is an experimental research design that examines the possible causes and effects between the treated group (experimental group) and the control group (not treated) and then compares the two (Pandia et al., 2018). Real experimental research is research that fully controls all factors that may interfere with internal validity so that this can provide stability to the results achieved as a treatment effect (Octiva et al., 2018). There are two factors that may interfere with internal validity, namely intrinsic factors which include changes in the individual or unit being studied that take place during the study (for example: history, maturity, testing, instrumentation, experimental mortality, statistical regression) and the second is extrinsic factors, namely the possibility

skewed research results as a result of differences in the selection of participants in the experimental group and the control group (Pandiangan et al., 2021). The existence of a control group and an experimental group chosen at random or haphazardly is the main characteristic of real experimental research (Pandiangan et al., 2018). Rambang or randomization is one way that can minimize the influence of extrinsic factors, the other way is called matching. This randomization and matching means to control the previously known variables, the method is to select an experimental group based on the same characteristics besides that, it can also be done by making the control and experimental groups the same in the relevant variables (Octiva et al., 2021). While the extrinsic factors can be done by holding a control group (Pandiangan, 2015).

The phytochemical test of the ethanolic extract of the red dragon fruit (*Hylocereus polyrhizus*) was conducted at the Pharmacy Laboratory of the Universitas Sumatera Utara. The maintenance of experimental animals, treatment, and animal surgery were carried out at the Biology

Laboratory of the Faculty of Mathematics and Natural Sciences (MIPA) of the Universitas Sumatera Utara. Preparation and reading of HE and CD40 immunohistochemistry of the heart of male wistar rats (*Rattus norvegicus*) were carried out at the Anatomical Pathology Laboratory, Faculty of Medicine, Universitas Sumatera Utara.

The population is the total number of units or individuals whose characteristics are to be studied (Pandiangan, 2018). The population of this study was male wistar rats (*Rattus norvegicus*) with a body weight of 150-200 grams, aged 3-4 months. The sample is part of the population studied in a study and the results will be considered an illustration of the original population, but not the population itself (Pandiangan et al., 2022). The research sample was taken randomly and divided into 7 groups, namely one control group who was given a normal diet, six treatment groups were given cooking oil 5 times, 10 times, and 15 times frying + ethanol extract of red dragon fruit (*Hylocereus polyrhizus*) at a dose of 60mg/kg BW and 90mg/kg BW orally.

Table 1: Research Design

Group	Number of Rats	Treatment	Treatment Time
K-0	5	Normal diet standard	60 Days
P-1	5	Cooking oil 5 times for frying + ethanol extract solution of red dragon fruit at a dose of 60mg/kg BW	60 Days
P-2	5	Cooking oil 5 times for frying + ethanol extract solution of red dragon fruit at a dose of 90mg/kg BW	60 Days
P-3	5	Cooking oil 10 times for frying + ethanol extract solution of red dragon fruit at a dose of 60mg/kg BW	60 Days
P-4	5	Cooking oil 10 times for frying + ethanol extract solution of red dragon fruit at a dose of 90mg/kg BW	60 Days
P-5	5	Cooking oil 15 times for frying + ethanol extract solution of red dragon fruit at a dose of 60mg/kg BW	60 Days
P-6	5	Cooking oil 15 times for frying + ethanol extract solution of red dragon fruit at a dose of 90mg/kg BW	60 Days

Data analysis used the Kruskal Wallis test and the Mann Whitney test. The Kruskal Wallis test is a ranking-based nonparametric test whose purpose is to determine whether there are statistically significant differences between two or more groups of independent variables on the dependent variable on a numerical data scale (interval/ratio) and an ordinal scale (Tobing et al., 2018). The Mann Whitney

test is a non-parametric test used to determine the difference in the median of 2 independent groups if the dependent variable data scale is ordinal or interval/ratio but not normally distributed (Tobing et al., 2018).

## RESULT AND DISCUSSION

### Result

**Results of Standardization of Ethanol Extract of Red Dragon Fruit (*Hylocereus polyrrhizus*)**

The results of standardization of red dragon fruit (*Hylocereus polyrrhizus*) ethanol extract can be seen in Table 2:

**Table 2: Results of Standardization of Red Dragon Fruit (*Hylocereus polyrrhizus*)**

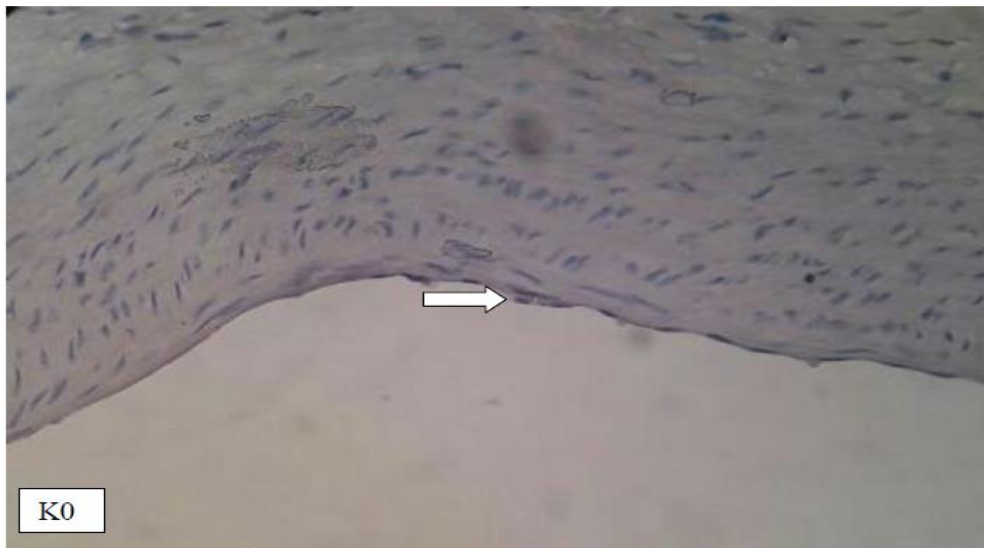
Characteristics	Results (%)	MMI Requirements (%)
Determination of water level	5.28 (Simplicity) 4.63 (Extract)	≤ 10
Determination of ash content	4.70	≤ 7.90
Determination of acid insoluble ash content	0.48	≤ 7.56
Determination of water soluble juice content	54.66	≥ 38.26
Determination of ethanol soluble extract content	42.91	≥ 41.36

Note: MMI = Materia Medika Indonesia

Based on the characteristics of the ethanolic extract of red dragon fruit (*Hylocereus polyrrhizus*) in Table 2, the water content of the extract has met the

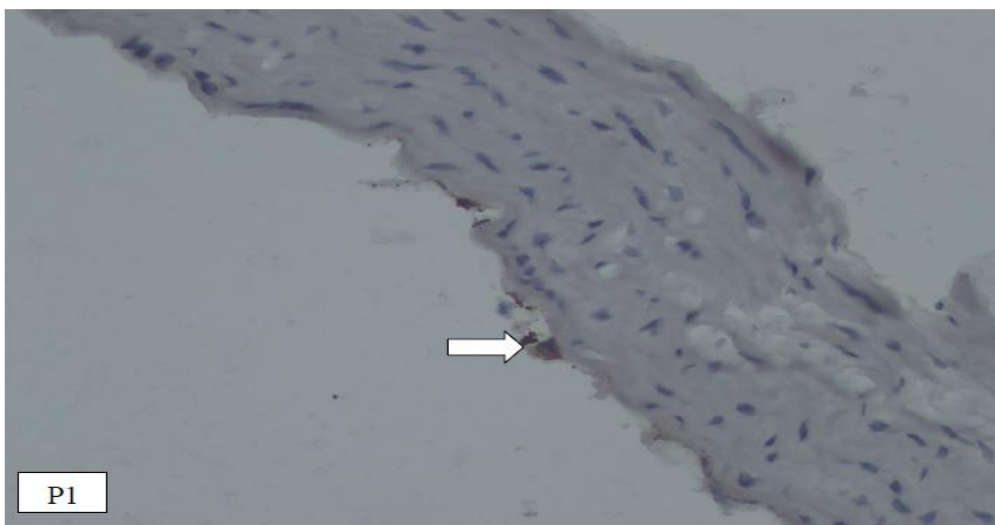
requirements of less than 10% because if the water content is more than 10% it can cause the growth of fungi, microbes, enzymatic reactions or hydrolysis processes.

**CD40 Immunohistochemical Overview of Cardiac Aorta of Male Wistar Rats**



Information: = Absence of CD40

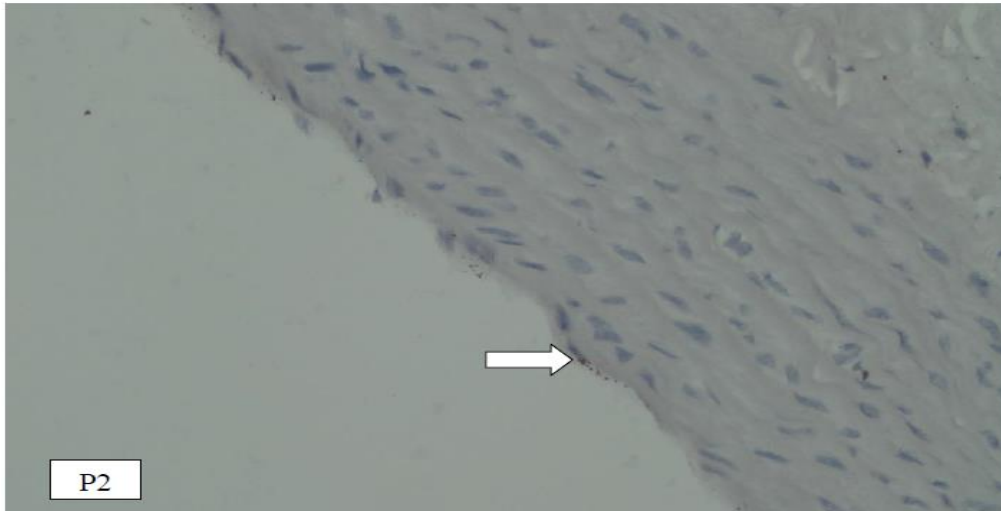
Figure 1: Immunohistochemical Overview of the Cardiac Aorta Control Group (K0) Male Wistar Rat, Magnification 400x



Information: = Presence of CD40

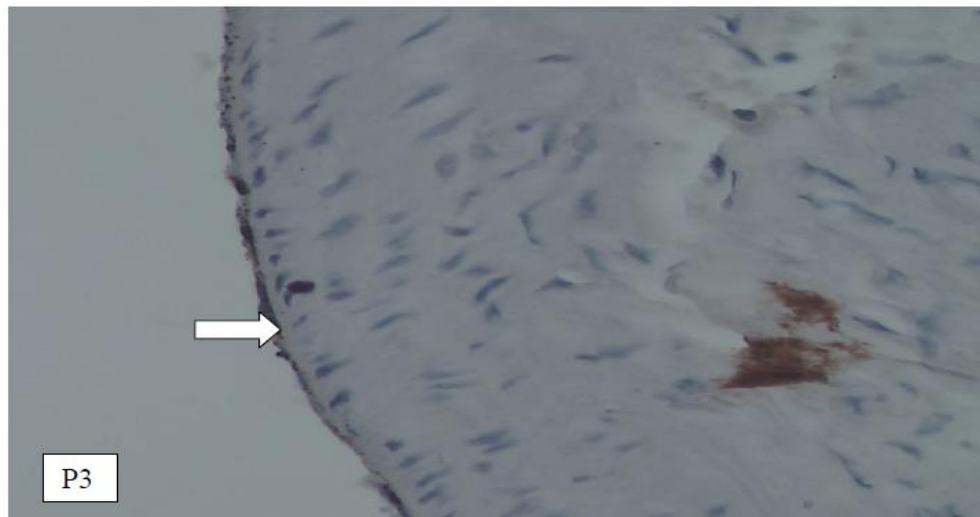
Figure 2: Immunohistochemical Description of Rat Heart Aorta CD40 Wistar Males Treatment Group (P1) Induced by Cooking Oil 5 Times Frying + Ethanol Extract of Red Dragon Fruit 60 mg/kg BW, Magnification 400x

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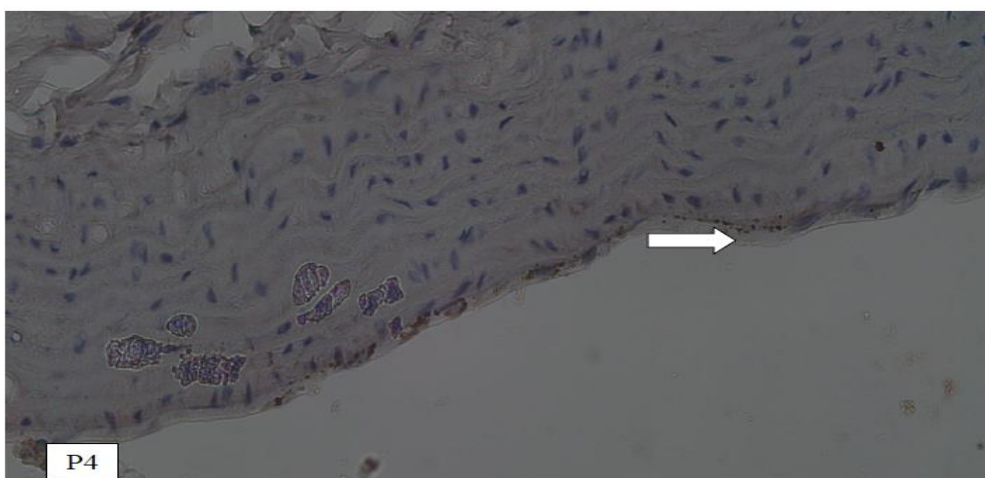
**Information:**  **Presence of CD40**

**Figure 3: Immunohistochemistry of CD40 Aortic Heart of Male Wistar Rats Treatment Group (P2) Induced by Cooking Oil 5 Times Frying + Ethanol Extract of Red Dragon Fruit 90 mg/kg BW, Magnification 400x**



**Information:**  **Presence of CD40**

**Figure 4: Immunohistochemical Description of CD40 Aortic Heart of Male Wistar Mice Treatment Group (P3) Induced by Cooking Oil 10 Times Frying + Ethanol Extract of Red Dragon Fruit 60 mg/kg BW, Magnification 400x**

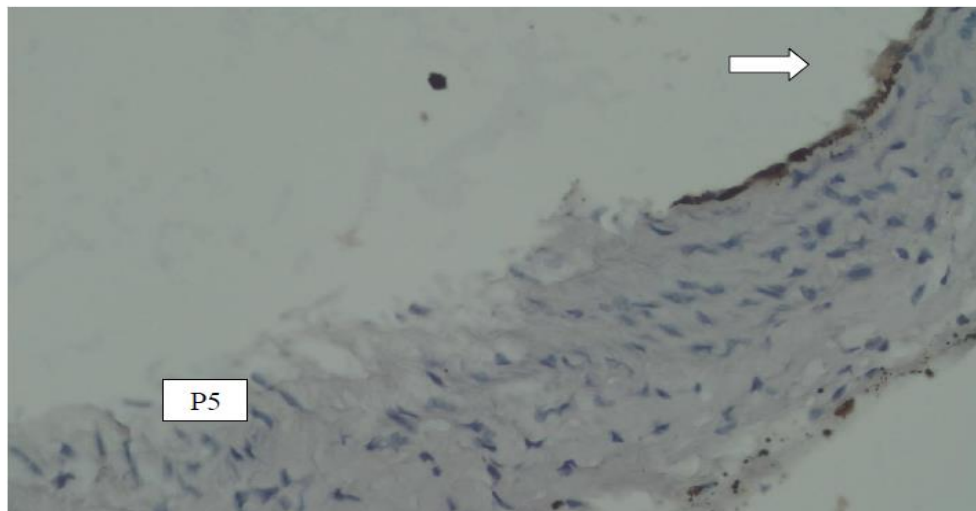


**Information:**  **Presence of CD40**

**Figure 5: Immunohistochemical Description of CD40 Aortic Heart of Male Wistar Rats Treatment Group (P4) Induced by Cooking Oil 10 Times Frying + Ethanol Extract of Red Dragon Fruit 90 mg/kg BW, Magnification 400x**

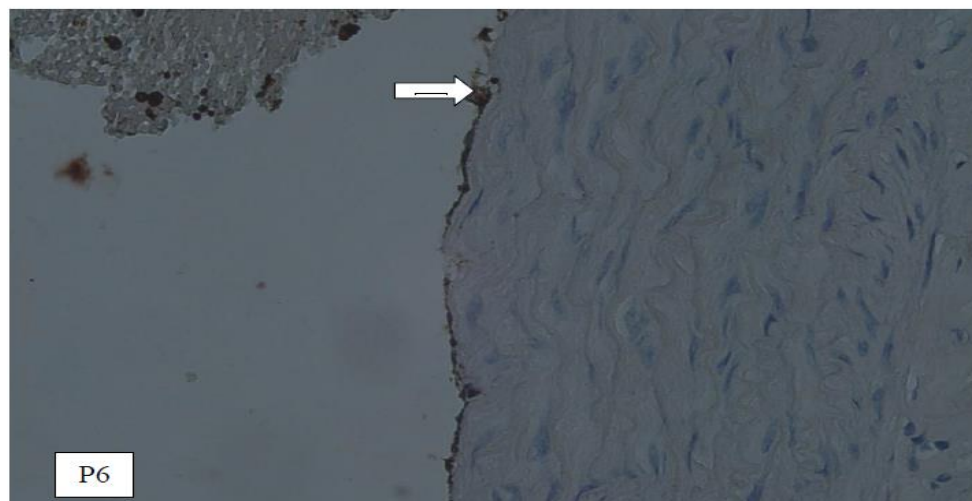
The results of the Mann Whitney test can be seen from Figure 1-7. Based on Figure 1, it is a normal picture of the heart aorta of male Wistar rats, namely on the

surface of the endothelial cells (arrows) no brown color is found as a marker for the absence of CD40/plaque.



Information: Presence of CD40

Figure 6: Immunohistochemical Description of CD40 Aortic Heart of Male Wistar Rats Treatment Group (P5) Induced by Cooking Oil 15 Times Frying + Ethanol Extract of Red Dragon Fruit 60 mg/kg BW, Magnification 400x



Information: Presence of CD40

Figure 7: Immunohistochemistry of CD40 Aortic Heart of Male Wistar Rats Treatment Group (P6) Induced by Cooking Oil 15 Times Frying + Ethanol Extract of Red Dragon Fruit 90 mg/kg BW, Magnification 400x

### Effect of EEBNM Administration on the Immunohistochemical Picture of CD40 Aortic Heart of Male Wistar Rats Induced by Cooking Oil 5 Times, 10 Times, and 15 Times Frying

Table 3: Kruskal-Wallis Test Results

Variable	P-Value
CD40 Staining Intensity	0.039

Based on the results of the Kruskal Wallis test, it was found that the value of p-

value=0.039 for CD40 staining intensity, it can be concluded that there was a significant difference (p-value<0.05) in the CD40 count between the treatment groups.

### DISCUSSION

Based on the results of the characteristics of red dragon fruit (*Hylocereus polyrhizus*) found the presence of flavonoid compounds. Flavonoids function as antioxidants, free radical

scavengers, hepatoprotective, anti-thrombotic, anti-inflammatory, and antiviral (Furhman et al., 2002). Flavonoids are phenolic compounds, a group of secondary metabolites that are most commonly found in plants. Phenolics have an aromatic ring A, one aromatic ring B and a middle ring containing oxygen and containing one or more hydroxyl groups. The hydroxyl group in phenolics has antioxidant activity and plays a role in capturing free radicals because the hydroxyl group plays a role in donating hydrogen atoms so that it can stabilize free radicals (Redha, 2010).

The results of this study showed that the administration of red dragon fruit (*Hylocereus polyrhizus*) ethanol extract containing flavonoids as antioxidants, anti-inflammatory, anti-thrombotic could reduce the CD40 count in the heart aorta of male Wistar rats induced by cooking oil 5 times, 10 times and 15 times. This is in accordance with research that a dose of 90 mg/kg BW can reduce the CD40 count. Anthocyanins are flavonoids found in red dragon fruit that can reduce the risk of atherosclerosis, blood pressure, inflammation and protect endothelial cell function (Teresa et al., 2010).

Monocyte chemotactic protein 1 (MCP-1) is a chemokine secreted by activated macrophages and endothelial cells in chronic, acute inflammatory disease and is directly involved in the development of atherogenesis. Anthocyanins play a role in protecting TNF- $\alpha$  inducing MCP-1 in human endothelial cells (Lampe and Chang, 2007), which can increase serum antioxidant capacity in hypercholesterolemic rabbits (Sharifiyan et al, 2016). Research conducted by administering anthocyanins to rats fed a high-fat diet showed a significant decrease in proinflammatory cytokines, namely TNF- $\alpha$ , MCP-1, and IL-10. Anthocyanins play a role in inhibiting the activity of the ACE (angiotensin-converting enzyme) enzyme which can reduce the inflammatory process and improve endothelial cell function (Hidalgo et al., 2012).

Anthocyanins as anti-inflammatory have a role in inhibiting CD40L, NF- $\kappa$ B, decreasing foam cell formation in the aorta of Wistar rats fed an atherogenic diet (Maharani et al., 2014), can reduce CD40 activation in endothelial cells caused by cholesterol distribution (Xia et al., 2007), can induce the expression of the enzyme Nrf2 (nuclear factor erythroid 2-related factor) which is a transcription factor as a defense against free radicals and binds to the antioxidant response (ARE). Activation of Nrf2-ARE plays a role in inhibiting the production of inflammatory mediators such as cytokines, chemokines, cell adhesion molecules so that it can reduce the pathogenesis of inflammation-related diseases such as atherosclerosis (Aboonabi et al., 2015).

## CONCLUSION AND SUGGESTION

The results of the Kruskal Wallis test showed that there was a significant difference between the seven treatment groups (p-value=0.039). The results of the Mann Whitney test in the treatment (P2) of frying 5 times at a dose of 90 mg/kg BW on the CD40 count the results showed a significant difference. This study proves that the ethanolic extract of red dragon fruit (*Hylocereus polyrhizus*) can reduce the number of CD40 in the heart aorta of male wistar rats induced by cooking oil.

It is necessary to conduct research on the active compounds of the ethanolic extract of red dragon fruit (*Hylocereus polyrhizus*) which contribute to CD40 reduction and further research on Nrf2-ARE which acts to inhibit the production of inflammatory mediators.

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**Conflict of Interest:** None

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**Ethical Approval:** Approved

## REFERENCES

1. Aboonabi, A. Singh, I. (2015). Chemopreventive Role of Anthocyanins in

- Atherosclerosis Via Activation of Nrf2-ARE as an indicator and Modulator of Redox. *Biomedicine and Pharmacotherapy Elsevier Masson SAS*, 72, 30–36. DOI: 10.1016/j.biopha.2015.03.008.
- Aini NN. (2012). Penggunaan Minyak Jelantah pada Pedagang Makanan Lesehan di Taman Kuliner Karang Malang. Laporan Penelitian Kesehatan Lingkungan.
  - Fuhrman B, Aviram M. (2002). Polyphenols and Flavonoids Protect LDL Against Atherogenic Modifications. *Handbook of Antioxidant*, 2nd edition. Marcel Dekker, Inc.
  - Hidalgo, M. Santamaria, MS. Recio, I. Sanchez-Moreno, C. Beatriz de Pascual-Teresa Gerald Rimbach, & Sonia de Pascual-Teresa. (2012). Potential Anti-Inflammatory, Anti-Adhesive, Anti-Estrogenic, And Angiotensin-Converting Enzyme Inhibitory Activities of Anthocyanins and Their Gut Metabolites. *Genes Nutr*, 7, 295-306.
  - Lampe JW & Chang JL. Interindividual Differences in Phytochemical Metabolism and Disposition. *Semin Cancer Biol*, 17, 347-53.
  - Maharani, T. Sargowo, D. Tjokropranowo, A. & Ratnawati, R. (2014). Effect of Extract Purple Ipomoe Batatas Cultivar Kawi Mountain Chronic Inflammation in Wistar Rats with Atherogenic Diet. *IEESE International Journal of Science and Technology (IJSTE)*, 3(1), 1-7.
  - Nindrea. (2015). Meta Analisis Faktor Risiko Penyakit Jantung Koroner di Asia Tenggara. Padang: Fakultas Kesehatan Masyarakat Universitas Andalas.
  - Octiva, C. S., Irvan, Sarah, M., Trisakti, B., & Daimon, H. (2018). Production of Biogas from Co-digestion of Empty Fruit Bunches (EFB) with Palm Oil Mill Effluent (POME): Effect of Mixing Ratio. *Rasayan J. Chem.*, 11(2), 791-797.
  - Octiva, Cut Susan, Indriyani, & Santoso, Ari Beni. (2021). Effect of Stirring Co-digestion of Palm Oil and Fruit for Biogas Production to Increase Economy Benefit. *Budapest International Research and Critics Institute-Journal*, 4(4), 14152-14160. DOI: <https://doi.org/10.33258/birci.v4i4.3521>.
  - Pandia, S., Tanata, S., Rachel, M., Octiva, C., & Sialagan, N. (2018). Effect of Fermentation Time of Mixture of Solid and Liquid Wastes from Tapioca Industry to Percentage Reduction of TSS (Total Suspended Solids). *IOP Conference Series: Materials Science and Engineering*, 309, 012086. DOI: 10.1088/1757-899X/309/1/012086.
  - Pandiangan, Saut Maruli Tua. (2015). Analisis Lama Mencari Kerja Bagi Tenaga Kerja Terdidik di Kota Medan. Skripsi. Medan: Fakultas Ekonomi dan Bisnis, Program Studi Ekonomi Pembangunan, Universitas Sumatera Utara. [https://www.academia.edu/52494724/Analisis\\_Lama\\_Mencari\\_Kerja\\_Bagi\\_Tenaga\\_Kerja\\_Terdidik\\_di\\_Kota\\_Medan](https://www.academia.edu/52494724/Analisis_Lama_Mencari_Kerja_Bagi_Tenaga_Kerja_Terdidik_di_Kota_Medan).
  - Pandiangan, Saut Maruli Tua. (2018). Analisis Faktor-faktor yang Mempengaruhi Penawaran Tenaga Kerja Lanjut Usia di Kota Medan. Tesis. Medan: Fakultas Ekonomi dan Bisnis, Program Studi Ilmu Ekonomi, Universitas Sumatera Utara. <http://repositori.usu.ac.id/bitstream/handle/123456789/10033/167018013.pdf?sequence=1&isAllowed=y>.
  - Pandiangan, Saut Maruli Tua, Rujiman, Rahmanta, Tanjung, Indra I., Darus, Muhammad Dho, & Ismawan, Agus. (2018). An Analysis on the Factors which Influence Offering the Elderly as Workers in Medan. *IOSR Journal of Humanities and Social Science (IOSR-JHSS)*, 23(10), 76-79. DOI: 10.9790/0837-2310087679.
  - Pandiangan, Saut Maruli Tua, Resmawa, Ira Ningrum, Simanjuntak, Owen De Pinto, Sitompul, Pretty Naomi, & Jefri, Riny. (2021). Effect of E-Satisfaction on Repurchase Intention in Shopee User Students. *Budapest International Research and Critics Institute-Journal*, 4(4), 7785-7791. DOI: <https://doi.org/10.33258/birci.v4i4.2697>.
  - Pandiangan, Saut Maruli Tua, Oktafiani, Fida, Panjaitan, Santi Rohdearni, Shifa, Mutiara, & Jefri, Riny. (2022). Analysis of Public Ownership and Management Ownership on the Implementation of the Triple Bottom Line in the Plantation Sector Listed on the Indonesia Stock Exchange. *Budapest International Research and Critics Institute-Journal*, 5(1), 3489-3497. DOI: <https://doi.org/10.33258/birci.v5i1.4016>.
  - Redha, A. 2010. Flavonoid: Struktur, Sifat, Antioksidatif, dan Perannya dalam Sistem Biologis. *Jurnal Bellan*, 9(2), 196-202.



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17. Robbins C. (2015). Inflammation and Repair, Pathologic Basis of Disease. Edisi Ke-9.
18. Sharifiyan, F., Attar, A., Nili, N., Asgary, S. (2016). Study of Pomegranate (*Punica granatum L.*) Peel Extract Containing Anthocyanins on Fatty Streak Formation in the Renal Arteries in Hypercholesterolemic Rabbits. *Advanced Biomedical Research*.
19. Siswanto W, Mulasari. (2015). Pengaruh Frekuensi Penggorengan Terhadap Peningkatan Peroksida Minyak Goreng Curah dan Fortifikasi Vitamin A. *Kesmas*, 9(1), 1-10.
20. Teresa, S.De Pascual, Moreno, D. A. & García-Viguera, C. (2010). Flavanols and Anthocyanins in Cardiovascular Health: aReview of Current Evidence. *International Journal of Molecular Sciences*, 11(4), 1679-1703. DOI: 10.3390/ijms11041679.
21. Tobing, Murniati, Afifuddin, Sya'ad, Rahmanta, Huber, Sandra Rouli, Pandiangan, Saut Maruli Tua, & Muda, Iskandar. (2018). An Analysis on the Factors Which Influence the Earnings of Micro and Small Business: Case at Blacksmith Metal Industry. *Academic Journal of Economic Studies*, 5(1), 17-23. <https://www.ceeol.com/search/article-detail?id=754945>.
22. World Health Organization. (2013). Deaths from Coronary Heart Disease. <http://www.who.int/cardiovascular-diseases/cvd14death-HD.pdf>.
23. Wierzbicki, As. (2007). Lipid-Altering Therapies and the Progression of Atherosclerotic Disease. *Cardiovasc Intervent Radiol*, 30, 155-160.
24. Wijaya. (2011). Pengaruh Ekstrak Kulit Buah Manggis (*Garcinia mangostana L*) Terhadap Penurunan Jumlah Foam Cell pada Aorta Tikus (*Rattus norvegicus*) Model Aterogenik. *Jurnal Universitas Brawijaya*, 2(1), 1-10.
25. Xia M, Ling W, Zhu H, Wang Q, Ma J, Hou M, Tang Z, Li L, &Ye Q. (2007). Anthocyanin Prevent CD40-Activated Proinflammatory Signaling in Endothelial Cells by Regulating Cholesterol Distribution. *Arterioscler Thromb Vasc Biol*, 27, 519-524.

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