

Peripheral Blood Profiles of CCl₄-Induced Wistar Rats after Date Seed Steeping Treatment

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Abstract: A various household appliances that containing CCl₄ can cause an inflammation. Utilization of date seed as an anti-inflammatory agent has never been done. The aim of the study was to explore the peripheral blood profiles of CCl₄-induced wistar rat after steeped date seed treatment. An experimental study, post-test only with control group design was used. Rats were assigned into 6 groups. There are negative control, positive control, healthy control and treatment groups (dose 1; 3; 5 g/kg). Data collected were neutrophils, lymphocytes and monocytes. The data was compared between the groups with the ANOVA test and was followed by post hoc test. Induction of CCl₄ causes an increase of monocytes, neutrophils and decreased lymphocytes number. Giving of dates seeds can reduce the number of monocytes and neutrophils but increase of lymphocytes number. A dose of 3 g/kg of dates seed has the same effect with a control group that given dexamethasone. The study showed for the first time anti-inflammatory of activity of steeped date seed can change the peripheral blood profile. Steeping of date seeds can repair tissue damage due to CCl₄ exposure to be marked by accelerating the inflammatory process.

1 BACKGROUND

Exposure to toxic compounds, microorganisms, tumors and trauma will lead to the onset of inflammatory process. The inflammatory process involves the role of peripheral blood cells to minimize damage. From the site of infection, chronic irritation and inflammation can also develop into various types of cancer. Lymphocytes and monocytes play an important role as predictor survival of several diseases (Li et al., 2014). The high levels of monocyte and/or neutrophil infiltration may be accompanied by cytotoxicity, angiostasis and tumor regression. The neutrophil-lymph node ratio (NLR) is an index indicating systemic inflammation (Unal et al., 2013). Lymphocyte-monocyte ratio (LMR) is a useful predictive factor for various cancers (Huang & Feng, 2015). The amount of eosinophil also correlates with inflammation in bronchial asthma (Hef et al., 2017; Katz et al., 2014). It suggests that the peripheral blood profile is sensitive to identify the status of systemic inflammation that occurs.

The peripheral blood features include eosinophil, neutrophil, basophil, lymphocytes and monocytes. Neutrophil acts as a bacterial phagocytosis and cell destruction with lysosome enzymes. Basophil plays an important role in hypersensitivity reactions. While eosinophil produces antihistamines and plays a role to prevent hypersensitivity reactions. Systemic inflammation responses play an important role in the progress of some diseases. Round blood examination is easy to do, accurate and low cost. Inflammation modifies the quality of the immune response. Lymphocytes and monocytes are the major immune cells in the inflammation response. The platelet-lymphocyte ratio is a critical sign and associated with a poor prognosis in some oncologic disorders (Gary et al., 2013).

Recently, several cross sectional and cohort studies have shown that the number of leukocytes is positively associated with the prevalence and incidence of metabolic syndrome (Sun et al., 2014). The use of drugs as anti-inflammation cause various side effects. One of the herb plants that allegedly act as immunomodulators is date seeds. The date seeds contain polyphenols (Ardekani et al., 2010) which

can function as antioxidants and useful for lowering blood cholesterol (Saryono, Eliyan, et al., 2017; Saryono, Rahmawati, et al., 2017) and glucose (El-Fouhil et al., 2011; El-Fouhil et al., 2010). However, there have been no studies that explain the peripheral blood parameters in inflammation due to CCl₄ toxicity given the steeping of date palms (*Phoenix Dactylifera* L.). Thus, this study aimed to determine the profile of peripheral blood in mice exposed to CCl₄ after being given the steeping of date seeds.

2 METHODS

2.1 Preparation of Dates

The matured seeds of *Phoenix dactylifera* were purchased from local market. Date palm seed of Deglet Nour was selected and washed with tap water, left to dry under the sun for one day. The dried date palm seed was roasted and ground with blender. Filtered the flour to obtain a fine powder.

2.2 Experimental Animal

Animal try in the form of white rat wistar grown male, age about 2-3 months with the weight range 150-200 grams. Rats were adapted for one week and placed in separate cages according to the group. All animals were given food and drink according to their needs (ad libitum), and kept on a light dark cycle 12/12 hours. They were conducted based on standardized protocols for experimental animal studies. This study followed the principles of laboratory established by Ethic Committee of Medical School, University of Sebelas Maret, Surakarta, Indonesia.

2.3 Research Design

The research was conducted using laboratory experimental, pre and post-test with control group design. The white rats were grouped into 6 groups, healthy control (HC), negative control (NC), positive control (PC), treatment dose 1 g/kg (T1), treatment dose 3 g/kg (T3), and treatment dose 5 g/kg (T5). Prior to treatment, all groups were induced with CCl₄ with a single dose of 0.2 ml/100g except group E6. The treatment of giving the date seeds was given for 14 days. Positive control using dexamethasone dose 0.5 mg/kg.

2.4 Statistical Analysis

The measured data were peripheral blood profiles including the absolute number of monocytes, eosinophil, neutrophil segments and stems, as well as lymphocytes with GIEMSA staining. The data obtained will be compared between groups with one way ANOVA test. The test results are said to be significant if $p < 0.05$. If there is a significant difference, the data are analysed further by the post hoc Least Significant Difference (LSD) test.

3 RESULTS

3.1 Phytochemical Test

Based on phytochemical test, steeping of date seed powder contain more flavonoid and tannin. Quantitative test results show that the steamed of date seeds powder contain flavonoids that is 20.68 mg/mL (Table 1).

3.2 Peripheral Blood Profile

The mean score of monocytes in the healthy control group was 1.8 ± 1.09 cells/mm³ while in the CCl₄-induced group 2 ± 0.76 cells/mm³. In the CCl₄-induced group, the number of monocytes increased but did not differ significantly with the healthy group. After the treatment period, the group given 5 mg/kg of date seeds had the greatest decrease and significant compared to the other groups. The lowest monocyte rate was 1.8 ± 0.44 in the treatment group with 5 mg/kg of date seeds (Table 2). Based on the Anova test, it was found that there was a difference of monocyte level between groups ($p = 0.000$). In the negative control group, the number of monocytes is still high. When compared with the positive control group, administering the seeds of dates can lower the number of monocytes larger. Based on the LSD post hoc test, treatment group given 3 g/kg and 5 g/kg duck seeds were not significantly different (Table 3), but generally the steeping of date palm seeds decreased the number of monocytes.

Table 1: Qualitative and quantitative test of phytochemical compound

No	Indicator	Level
Qualitative test		
1	Flavonoid	+++
2	Tannin	+++
3	Saponine	++
4	Alkaloid	+
Quantitative test		
1	Polyphenol level	8.44 mg/mL
2	Flavonoid level	20.68 mg/mL

The mean number of lymphocytes in the healthy group was 40.8 ± 2.58 cells/mm³, whereas in the

induction group CCl₄ was 37.04 ± 2.75 cells/mm³. There was a significant difference in the number of lymphocytes between the healthy group and the CCl₄ induced group ($p < 0.009$). After the treatment period, the average number of lymphocytes increased (Table 4). Anova test results showed that there were significant differences in the number of lymphocytes between groups ($p = 0.000$). The greatest increase occurred in the positive control group given dexamethasone, and did not differ significantly with the group given dose 3 g/kg (Table 4). Based on the LSD post hoc test, the group given doses of 1 and 5 g/kg date seeds were not significantly different.

Table 2: Number of monocytes, lymphocytes and neutrophils before and after administration of dates seed steeping

Groups	Monocytes				Lymphocytes				Neutrophils			
	Pre test		Post test		Pre test		Post test		Pre test		Post test	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
HC	1.80	1.09	1.40	0.54	40.80	2.58	36.40	2.30	57.2	2.38	60.8	3.27
NC	1.40	0.54	6.00	1	38.40	2.70	23.80	3.03	58.4	2.88	59.2	3.42
PC	2.20	0.83	2.80	0.83	38.60	2.40	40.60	2.60	57.6	2.96	52.6	2.96
T1	2.20	0.44	3.00	1	37.40	2.96	36.00	1.22	58.6	2.70	57.6	2.07
T3	1.80	0.83	2.40	0.54	35.40	1.51	37.00	4.00	61.6	1.94	55.4	3.64
T5	2.40	0.89	1.80	0.44	35.40	2.96	34.60	2.70	59.8	1.92	58.8	2.28
Anova test	p = 0.000				p = 0.000				p = 0.03			

Table 3: LSD test results of monocyte count after treatment

Group	HC	NC	PC	T1	T3	T5
HC	-	0.000	0.000	0.000	0.000	0.000
NC	0.000	-	0.683	0.416	0.049	0.008
PC	0.000	0.683	-	0.226	0.020	0.003
T1	0.000	0.416	0.226	-	0.226	0.049
T3	0.000	0.049	0.020	0.226	-	0.416
T5	0.000	0.008	0.003	0.049	0.416	-

*: Significant ($p < 0.05$)

Table 4: LSD Test results on the number of lymphocytes after treatment

Group	HC	NC	PC	T1	T3	T5
HC	-	0.000	0.000	0.000	0.000	0.000
NC	0.000	-	0.015	0.051	0.002	0.025
PC	0.000	0.015*	-	0.574	0.432	0.821
T1	0.000	0.051*	0.574	-	0.184	0.735
T3	0.000	0.002*	0.432	0.184	-	0.315
T5	0.000	0.025	0.821	0.735	0.315	-

*: Significant ($p < 0.05$)

In the early stages of inflammation, neutrophils have increased, while subsequently replaced by monocytes. After induction of CCl₄, neutrophils had a slight increase with mean of 59.20 ± 2.70

cells/mm³, while in the healthy group 57.2 ± 2.38 cells/mm³. There was no difference in the number of neutrophils between the CCl₄-induced group and the healthy control group ($p > 0.05$). After the treatment

period, there was an average difference between mature neutrophil levels between groups ($p = 0.03$). Based on the LSD post hoc test, the positive control

group differed significantly with the treatment group, but between treatment groups treated with dates did not differ (Table 5).

Table 5: LSD Test result on the number of neutrophil after treatment

Group	HC	NC	PC	T1	T2	T3
HC	-	0.002	0.407	0.057	0.835	0.407
NC	0.002	-	0.014	0.153	0.003	0.000
PC	0.407	0.014	-	0.258	0.533	0.105
T1	0.057	0.153	0.258	-	0.086	0.009
T3	0.835	0.003*	0.533	0.086	-	0.302
T5	0.407	0.000*	0.105	0.009	0.302	-

*: Significant ($p < 0.05$)

Based on the analysis of the overall data, giving positive control (dexamethasone) showed no difference with the giving of dill seeds dose 3 g/kg. This means that the steeping dose of 3 g/kg doses is proportional to the effects of dexamethasone drugs as anti-inflammatory.

4 DISCUSSION

Epidemiological studies have shown that peripheral blood profiles play a role in inflammation. CCl₄ toxicity can cause inflammation, but the mechanism of using dates as anti-inflammatory in CCl₄ toxicity is unclear. Giving CCl₄ effect on the occurrence of acute inflammation, especially liver and kidney organ. The number of monocytes and neutrophils increased, while the number of lymphocytes decreased after CCl₄ induction. These occur because the inflammation caused spur the secretion of monocytes and neutrophils to fight inflammation. The presence of trauma to the cells will trigger an inflammatory response, but some factors can boost immunity so that it can reduce inflammation (Viana et al., 2014).

In chronic inflammation, an increase in monocytes in peripheral blood. After 24 hours, the monocyte goes to the tissues and forms macrophages. Macrophages will be activated by TNF- α and activate iNOS (inducible nitrite oxide synthase) and produce NO. The CCl₄ compound that enters the liver will be converted to CCl₃ \cdot . CCl₃ \cdot bonding with NO will lead to new, more toxic compounds, peroxynitrite (ONOO \cdot). The ONOO \cdot compound will cause endothelial poisoning, resulting in decreased endothelial NO production. This causes vasoconstriction so that the inflammation gets worse. Giving dates flavonoids (Yasin et al., 2015; Mohamed et al., 2014), can act

as antioxidants, by donating H⁺ to CCl₃ \cdot to become neutral. This is what causes the number of monocytes to return to normal.

Neutrophils are innate immunity components that are abundant in the circulation. Neutrophils play a role in the fight against invasive pathogens, although it can also increase certain inflammatory diseases. At the time of acute inflammation there is an increase in the number of neutrophils that will degrade the phospholipid membrane and form arachidonic acid. With the help of the enzyme cyclooxygenase-2 (COX2), arachidonic acid is converted to prostaglandin. An increase in the number of neutrophils is activated by IL-1 β pro-inflammatory cytokines. Along with cell repair by the body's immune system, the number of neutrophils will decrease. Some types of diseases accompanied by high neutrophils have more severe clinical outcome problems than controls such as pain, bleeding, infarction and death (Zhang et al., 2016). The results showed that neutrophil recruitment to the site of infection was enhanced by serotonin delivered by platelets in innate immune reactions (Duerschmied et al., 2017). Several studies have clarified the association of neutrophils with prognostic diseases (Hashimoto et al., 2013; Li et al., 2014; Jiang et al., 2015).

Increasing the number of lymphocytes at a time of chronic inflammation is due to the immune system trying to form antibodies as a defense against CCl₄ radicals. But after the seeding of the date palm, the free radicals CCl₃ \cdot decreases and the lymphocytes return to normal. Baldness dates palm seeds contain many flavonoids that can act as antioxidants (Tang et al., 2013). This is evident from previous research that the steeping of date palm seeds can reduce malondialdehyde as a free radical oxidation product and increase antioxidant enzymes (Saryono, Hisni, et al., 2017). High leukocyte content, neutrophil count and high neutrophil-

lymphocyte ratios correlated with failure of ST elevation myocardial infarction (STEMI). This indicator is a good failure predictor of the case (Ghaffari et al., 2014). Other studies have shown that leukocytes play a role in the generation of oxidative stress and inflammation in diabetic mellitus patients (Shurtz-Swirski et al., 2001).

5 CONCLUSIONS

CCl₄ toxicity can lead to visible inflammation in the presence of an increase in the number of monocytes, neutrophils and decreased lymphocytes. Giving of dates seeds can reduce the number of monocytes, neutrophils and increase lymphocytes. Steeped of palm seeds can repair tissue damage due to CCl₄ exposure in animals that to be marked by accelerating the inflammatory process. The date seed steeped of 3 g/kg have a comparable to the effects of the drug dexamethasone as anti-inflammatory.

Implication: The use of date palm seed as anti-inflammatory can be given in the form of steeping. The first study to look at the effects of date seed as anti-inflammatory based on peripheral blood profiles can be used as an early reference for further research.

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