ARTICLE

THE EFFECT OF GREEN TEA IN REDUCING LIVER INJURY CAUSED BY METHOTREXAT WHICH INDICATED BY THE DECREASING OF ALANINE TRANSAMINASE (ALT) OF BALB/C MICE

Submitted to fulfil the assignment
And fit-out requisite in passing
Undergraduate Education Program
Medical Faculty

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FACULTY OF MEDICINE

DIPONEGORO UNIVERSITY

SEMARANG

2006

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TABLE OF CONTENT

THE EFFECT OF GREEN TEA IN REDUCING LIVER INJURY CAUSED BY METHOTREXAT WHICH INDICATED BY THE DECREASING OF ALANINE TRANSAMINASE (ALT) OF BALB/C MICE

Alfina Sorida[1], pudjadi [2]

ABSTRACT

Background: Cathecin, polyphenols within green tea has been studied can prevent liver injury, indicating in decreased ALT serum in blood. Methotrexate is an antimetabolite used in certain neoplastic diseases treatment, causes severe side effect as hepatotoxicity, fibrosis. In vivo studies are needed to examine the green tea liver injury-preventive effect that caused by methotrexate.

Objective: The purpose of this study was to analyze whether the consumption of 46.8 mg/ day green tea for 25 days can reduce liver injury which induced with 0.065 mg/ day methotrexate for the last 5 day experiment measured with alanine aminotransferase (ALT) test.

Design: Healthy male Balb/c mice (20-30 g body weight, 6-8 wk old) were used. A dose of methotrexate (0.065 mg/ day) was administered orally to induce liver injury and green tea (46.8 mg/ day) was administered orally by orogastric tube. The blood sample was taken on day-32 from *plexus venousus retro orbitalis* to assess the serum levels of alanine aminotransferase (ALT).

Results: The consuming of 46.8 mg/ day green tea for 25 days not significantly proved prevent liver injury in mice that induced with 0.065 mg/ day methotrexate for the last 5 day experiment. Analysis result was considered not significant.

Conclusions: Analysis result was considered not significant.

Key Words: cathecin • green tea • methotrexate • liver injury • ALT serum

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Pengaruh Pemberian Teh Hijau *(Camellia sinensis)* Terhadap Jumlah ALT Mencit Balb/c yang diberi Metotreksat

Alfina Sorida [1], Pudjadi [2]

ABSTRAK

Latar belakang : Katekin, salah satu dari senyawa polifenol teh hijau telah diteliti dapat menghambat kerusakan hepar yang ditandai dengan penurunan serum ALT di dalam darah. Metotrexat adalah obat antimetabolit yang biasa dipakai dalam pengobatan kanker dengan efek samping hepatotoksik dan fibrosis hepar. Penelitian lebih lanjut diperlukan untuk memeriksa efek teh hijau dalam menghambat kerusakan hepar yang disebebkan oleh metotrexat.

Objektif: tujuan penelitian ini adalah untuk mengatahui apakah pemberian teh hijau dosis 46,8 mg/hari selama 25 hari dapat menghambat kerusakan hepar pada mencit Balb/c yang beri diberi metotreksat dosis 0,065 mg/hari selama 5 hari terakhir yang dinilai dengan serum ALT.

Metode: penelitian ini menggunakan hewan percobaan mencit sehat umur 6-8 minggu dengan berat 20-30 gram. Metotrexat diberikan 0.065 mg per hari secara oral untuk memberikan efek kerusakan hepar. Sedangkan tehijau diberikan secara oral dengan dosis 46,8 mg per hari. Sampel darah diambil pada hari ke-32 melalui *plexus venousus retro orbitalis* untuk menghitung serum ALT.

Hasil : Pemberian teh hijau dosis 46,8 mg/hari selama 25 hari tidak terbukti dapat menghambat kerusakan hepar pada mencit Balb/c yang beri diberi metotreksat dosis 0,065 mg/hari selama 5 hari terakhir.

Kesimpulan : Pemberian teh hijau dosis 46,8 mg/hari selama 25 hari tidak terbukti dapat menghambat kerusakan hepar pada mencit Balb/c yang beri diberi metotreksat dosis 0,065 mg/hari selama 5 hari terakhir. Hasil ini menunjukkan bahwa tidak terdapat perbedaan yang bermakna pada ketiga kelompok.

Kata kunci : katekin, Teh hijau, metotreksat, kerusakan hepar, serum ALT

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Introduction

Green tea is particularly rich in polyphenols, including catechins, theaflavins and thearubigins, which are thought to contribute to the health benefits of tea.[1-9] Epidemiological surveys have associated tea drinking with reduced risk of cardiovascular diseases (CVD) and cancer. [1-4] Previous study reported that catechin in green tea have mechanism of the liver injury-preventive effect. It also has been investigated that the minimum green tea dose for significant effect result of ALT activities in reducing the liver injury in human was 10 cups per day.[10]

Methotrexate is an antimetabolite used in the treatment of certain neoplastic diseases, severe psoriasis, and adult rheumatoid arthritis. Methotrexate inhibits dihydrofolic acid reductase. Dihydrofolates must be reduced to tetrahydrofolates by this enzyme before they can be utilized as carriers of one-carbon groups in the synthesis of nucleotides and thymidylate. Therefore, methotrexate interferes with DNA synthesis, repair, and cellular replication. Actively proliferating tissues such as malignant cells, bone marrow, fetal cells, buccal and intestinal mucosa, liver cells and cells of the urinary bladder are in general more sensitive to this effect of methotrexate. Methotrexate causes hepatotoxicity, fibrosis and cirrhosis. Acutely, liver enzyme elevations are frequently seen.[11]

The present study was undertaken to investigate green tea capability to reduce liver injury which induced by methotrexate that indicates by alanine aminotransferase (ALT) test in mice.

Materials and Methods

Chemicals and Reagents. Dose was converted for 70 kg weight of human into 20 gram mice, which resulted in 0.0026.[12] Methotrexat was taken from Kimia Farma. Dose for human is 25-30 g methotrexat per day in order to have a significant effect for ALT activities. This experiment used 25 g methotrexat converted into mice which resulted in 0.065 mg. It poured into 50 cc of water, and it was given 0.5 cc per day with orogastric tube. Mice were given 6.5 mg methotrexat in order to have 0.065 mg methotrexat in 0.5 cc. green tea was obtained from market, branded and produced by PT Duta Serpack Inti Tangerang, named 2 Tang. This experiment used dose 10 cups green tea per day. Each cup needed 18 g per day for human, which converted into mice which resulted in 46.8 mg. It poured into 100 cc of water, and it was given 0.5 cc per day with orogastric tube. Mice were given 9.4 g green tea in order to have 46.8 mg green tea in 0.5 cc.

Animal Experiments. In the first week, 15 healthy 6-8 weeks-aged male Balb/c mice (20-30 g) were fed with the standard diets *ad-libitum* in order to adapt the new environment. In the second week mice were divided randomly into 3 groups. Each group contained 5 mice. Each group was caged separately in Biochemistry Laboratory of Medical Faculty of Diponegoro University Semarang. Starting from the second week, first group (control group) was fed and watered *ad-libitum* , second group (P1) was fed with the standard diets and was given methotrexat per oral (0.065 mg) started from day-27 till day-31, third group (P2) was fed with the standard diets supplemented by the green tea (46.8 mg) for 25 days started from day-8 till day-32 and was given methotrexat per oral (0.065mg) started from day-27 till day-31. The blood sample was taken on day-32 from *plexus venousus retro orbitalis* and measured by Dimension clinical chemistry

system produced by DADE BEHRING. The blood assay kit was obtained from Clinical Pathology Laboratory of Medical Faculty of Diponegoro University Semarang.

ALT Analysis. First, bloods from 6-8 weeks-aged male Balb/c mice (20-30 g) were prepared by the standard differential centrifugation technique. Bloods were measured by kit with some procedures. Alanin Aminotransferase (ALT) catalyzed the transamination from L- Alanin to α-ketoglutarate, forming L-glutamate and pyruvat. The pyruvat formed was reduced to lactate by lactate dehydrogenase (LDH) with simultaneous oxidation of reduced nicotinamide adenine dinucleotide (NADH). The change in absorbance with time due to the conversion of NADH to NAD was directly propotional to the ALT activity and was measured using a bichromatic (340-700 nm) rate technique.

Data Analysis. Data was processed using a SPSS program SPSS version 13.0 for windows.

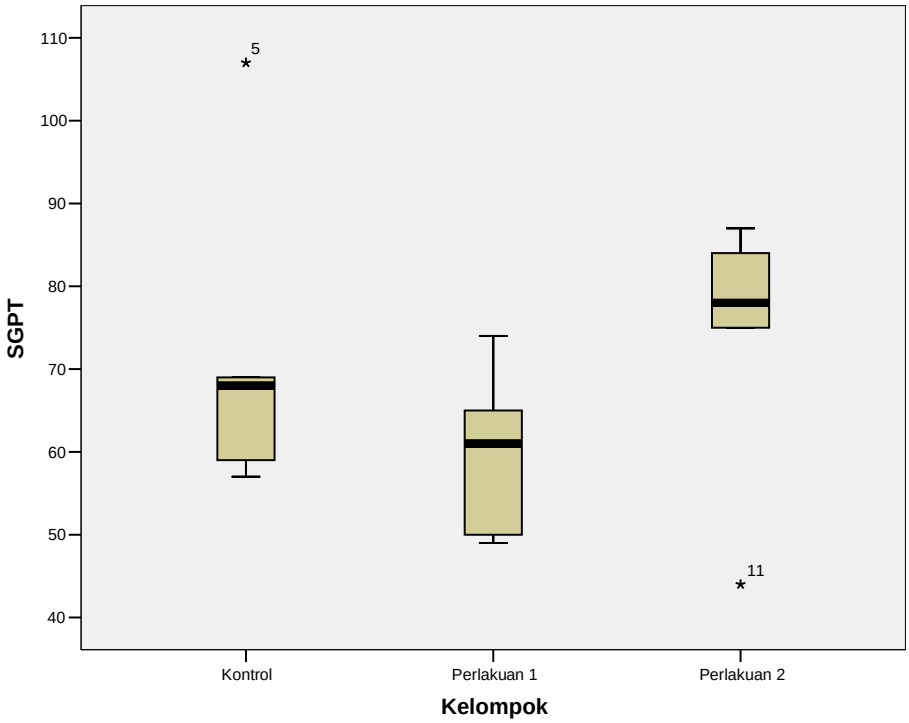
Results

Table1. Result of ALT analysis

Group	sample (n)	Median (U/L)	Min (U/L)	Max (U/L)
Control	5	68	57	107
Group 1 (P1)	5	61	49	74
Group 2 (P2)	5	78	44	87

The lowest average of ALT serum was 59.8 from group 1 and the highest one was 73.6, came from group 2.

Box plot data:



Data were tested using *Saphiro- Wilk* normality test and considered not normal ($P < 0.05$). Then data processing continued with *Kruskal - Wallis* non parametric statistic test which resulted in 0.264. It means that there was no significant change in the activities of serum ALT ($P > 0.05$)

Discussion

Analysis result was considered no significant. This result was different from several previous experiments which were resulted green tea could prevent liver injury.[10,13] In this study we used the brewed green tea which was given orally in order to have easier, cheaper, and less risk methods. In spite of several succeed studies did the same method, not using the extract cathecine, which is known as a active substrate and also the orally administration that could not be absorbed 100 per cent, could give the reasons of this result. Liver injury, the side effect of methotrexate also did not occur in this experiment. This experiment assumed that lack of dose and less term of methotrexate administration did not toxic enough to emerge a liver injury. [14, 15]

The consuming of 46.8 mg/ day green tea for 25 days not significantly proved prevent liver injury in mice which induced with 0.065 mg/ day methotrexate for the last 5 day experiment.

Suggestion

Therefore in the future more studies on the green tea effect to prevent liver injury which caused by methotrexate must be done by using the precise dose, term and materials. These studies are needed for better understanding in the green tea liver injury-preventive effect.

Acknowledgment

We thank Dr. Pudjadi SU, Dr. Andrew Johan M. Si and Dr. Tri Indah Winarni for helpful suggestions in the preparation and supervision of this manuscript. Staff of Pathology Clinic Unit Dr. Karyadi Hospital. Mr. Dukut and Mr. Sucipto in contributing the mice for this experiment.

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APPENDIX

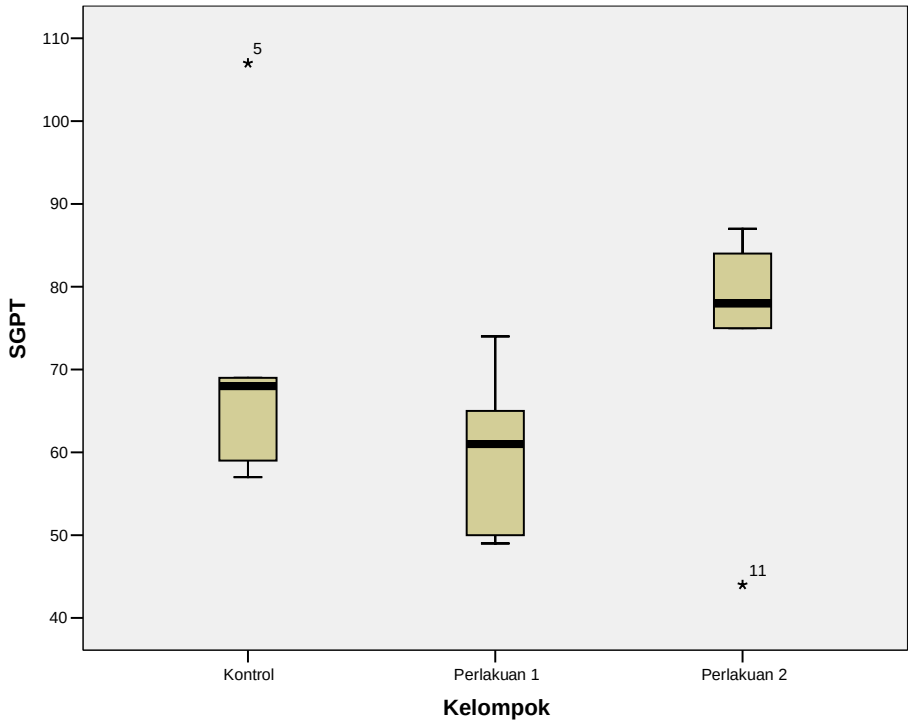
Case Processing Summary

		Cases					
		Valid		Missing		Total	
	Kelompok	N	Percent	N	Percent	N	Percent
SGPT	Kontrol	5	100.0%	0	.0%	5	100.0%
	Perlakuan 1	5	100.0%	0	.0%	5	100.0%
	Perlakuan 2	5	100.0%	0	.0%	5	100.0%

Descriptives

	Kelompok			Statistic	Std. Error
SGPT	Kontrol	Mean		72.00	9.066
		95% Confidence Interval for Mean	Lower Bound	46.83	
			Upper Bound	97.17	
		5% Trimmed Mean		70.89	
		Median		68.00	
		Variance		411.000	
		Std. Deviation		20.273	
		Minimum		57	
		Maximum		107	
		Range		50	
		Interquartile Range		30	
		Skewness		1.861	.913
		Kurtosis		3.693	2.000
	Perlakuan 1	Mean		59.80	4.705
		95% Confidence Interval for Mean	Lower Bound	46.74	
			Upper Bound	72.86	
		5% Trimmed Mean		59.61	
		Median		61.00	
		Variance		110.700	
		Std. Deviation		10.521	
		Minimum		49	
		Maximum		74	
		Range		25	
		Interquartile Range		20	
		Skewness		.288	.913
		Kurtosis		-1.449	2.000
	Perlakuan 2	Mean		73.60	7.698
		95% Confidence Interval for Mean	Lower Bound	52.23	
			Upper Bound	94.97	
		5% Trimmed Mean		74.50	
		Median		78.00	
		Variance		296.300	
		Std. Deviation		17.213	
		Minimum		44	
		Maximum		87	
		Range		43	
		Interquartile Range		26	
		Skewness		-1.823	.913
		Kurtosis		3.561	2.000

ALT/ SGPT



Non Parametric Tests

Kruskal-Wallis Test

Ranks

	Kelompok	N	Mean Rank
SGPT	Kontrol	5	8.20
	Perlakuan 1	5	5.60
	Perlakuan 2	5	10.20
	Total	15	

Test Statistics[a,b]

	SGPT
Chi-Square	2.660
df	2
Asymp. Sig.	.264

a. Kruskal Wallis Test

b. Grouping Variable: Kelompok