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- Efek Teh Hijau Terhadap Peningkatan Fluiditas Darah dan Penurunan Berat Badan
- Koherensi Remaja Pecandu Miras dalam Konteks *Salutogenesis*
- Pengetahuan, Sikap, dan Praktik Kewaspadaan Universal Perawat terhadap Penularan HIV/AIDS
- *Bedside Teaching*: Apakah Metode ini Efektif pada Pembelajaran Klinik Mahasiswa Keperawatan?
- Efek Orlistat, Ekstrak Biji Kopi Hijau, dan Kombinasinya terhadap Kadar Adiponektin dan Profil Lipid
- Peranan Kebersihan Kulit Kepala dan Rambut dalam Penanggulangan Epidemiologi *Pediculus Humanus Capitis*
- Deteksi Keparahan Fungsi Ginjal Melalui Perubahan Kritis Laju Filtrasi Glomerulus Pasien Hemodialisa
- Pengetahuan, *Self Efficacy* dan Stres Pasien Kusta melalui Penerapan *Support Group* dengan Pendekatan Teori Adaptasi
- Posisi Lateral Kiri Elevasi Kepala 30 Derajat terhadap Nilai Tekanan Parsial Oksigen (PO<sub>2</sub>) pada Pasien dengan Ventilasi Mekanik
- Intervensi Psikoedukasi Meningkatkan Kemampuan Pralansia dalam Penanganan Hipertensi
- Pengembangan Skala Kualitas Asuhan Keperawatan untuk Anak di Rumah Sakit dengan Infeksi Saluran Pernafasan Akut di Indonesia
- Pengembangan Model Pelayanan Asuhan Keperawatan Bayi Berat Lahir Rendah
- *Spiritual Emotional Freedom Technique* (SEFT) Menurunkan Stres Pasien Kanker Serviks
- Kontrol Diri dan Efikasi Diri Meningkatkan Kepuasan Ibu Menjalani Proses Persalinan
- Produksi ASI Ibu *Post Sectio Cesareae* dengan Pijat Oketani dan Oksitosin
- Tingkat Nyeri dan Prostaglandin-E2 pada Ibu Inpartu Kala I dengan Tindakan *Counter-pressure*
- Prediksi Penderita Gangguan Jiwa Dipasung Keluarga
- Keperawatan Spiritualitas pada Pasien Skizofrenia
- *Ethnonursing* Penggunaan Terapi Komplementer pada Suku Using Banyuwangi
- Kondisi Ekonomi dan Budaya Keluarga dengan Status Gizi Balita
- *Self Management Intervention* sebagai Upaya Peningkatan Kepatuhan pada Penderita DM
- Makna Seksualitas pada Remaja Etnis Jawa di SMA Wilayah Surabaya Utara

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## Table of Contents

No.	Title	Page
1	<b>The Impact of Green Tea on Blood Fluidity Improvement and Weight Loss</b>	1 - 5
2	Sense of Coherence on Liquor Abuse among Teenagers in Salutogenesis Context	5 - 10
3	Nurses` Knowledge, Attitudes, and Practices of Universal Precaution toward HIV/AIDS Transmission	11 - 18
4	Bedside Teaching: Is it Effective Methods in Clinical Nursing Students Learning?	19 - 25
5	The Effect of Orlistat, Green Coffee Bean Extract, and Its Combinations on Lipid Profile and Adiponectin Levels	26 - 34
6	The Importance Of Hair And Scalp Hygiene For Pediculus Humanus Capitis Epidemic Prevention	35 - 42
7	Severity Renal Function Detection through Critical Changes Glomerular Filtration Rate in Hemodialysis Patients	43 - 48
8	Knowledge, Self Efficacy and Stress in Patient With Leprosy Through Implementation of Support Group Using Adaptation Theory	49 - 58
9	Left Lateral Positioning with Head Elevation Increase The Partial Pressure of Oxygen on Patients with Mechanical Ventilation	59 - 65
10	Psychoeducation Intervention influence on the ability of the Pre-Senile Hypertension management	66 - 73
11	Development of the Quality of Nursing Care Scale for Hospitalized Acute Respiratory Infection Children in Indonesia	74 - 82
12	The Development of Nursing Care Services Model for Low Birth Weight Infants	83 - 90
13	Spiritual Emotional Freedom Technique Decreasing Stress on Patiens with Cervical Cancer	91 - 96
14	Self Control and Self Efficacy Increase Motherâ€™s Birth Labor Satisfaction	97 - 103
15	Breastmilk Production of Mother with Post Caesarean Section Given Oketani and Oxitocyn Massage	104 - 110
16	Pain Level and Prostaglandin-E2 using Counter-pressure during 1st Stage of labor	111 - 117
17	Prediction of Mental Disorders Deprived by Family	118 - 125
18	Spirituality Nursing among Patients with Schizophrenia	126 - 132
19	Ethnonursing for Utilizing Complementary Therapy at Using Tribes in Banyuwangi, East Java, Indonesia	133 - 137
20	Familyâ€™s Economic Level and Culture Correlate with Nutritional Status of Children Under Five Years	138 - 142
21	Self Management Intervention increasing compliance in patient with DM	143 - 150
22	The Meaning of Sexuality for Javaneese Adolecent at Senior High School, North Surabaya	151 - 157

## The Impact of Green Tea on Blood Fluidity Improvement and Weight Loss

### Efek Teh Hijau Terhadap Peningkatan Fluiditas Darah Dan Penurunan Berat Badan

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#### Abstract

**Introduction:** Various studies have shown the beneficial effects of green tea, not only on cardiovascular diseases but also on type 2 diabetes. **Method:** In this study, the preparation of green tea water extract has been standardized to (-)-epigallocatechin gallate (EGCG), the major component of green tea. The role of green tea water extract on blood fluidity and diabetes diseases has been studied in 13 Fructose-Fed Rat (FFR). The rats were given high fructose diet ad libitum for one week and then combination with green tea water extract every day for 6 days. **Results:** The results show, green tea water extract can reduces 100  $\mu$ L blood passage times of wistar rat significantly ( $p < 0.01$ ) by Micro-Channel Array Flow Analyzer (MC-FAN) instrument. Green tea water extract also had strong effect in reducing abdominal fat ( $p < 0.05$ ), blood glucose level ( $p < 0.01$ ) and body weight ( $p < 0.01$ ). **Discussion:** These results suggest that green tea water extract may has beneficial effects for the treatment of diabetes and reduce blood viscosity

**Keyword :** green, tea, epigallocatechin, gallate, HPLC, blood, fluidity, fructose-fed, rat,

#### Daftar Pustaka :

1. **Beltz LA, Bayer DK, Moss AL, Simet IM, (2006).** Mechanisms of cancer prevention by green tea and black tea polyphenols. - : Anticancer Agent Med Chem
2. **Lin YS, Tsai YJ, Tsay JS, Lin JK, (2003).** Factors affecting the levels of tea polyphenols and caffeine in tea leaves. - : J Agric Food Chem

# EFEK TEH HIJAU TERHADAP PENINGKATAN FLUIDITAS DARAH DAN PENURUNAN BERAT BADAN

## *(The Impact of Green Tea on Blood Fluidity Improvement and Weight Loss)*

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### ABSTRAK

**Pendahuluan:** Berbagai penelitian telah menunjukkan efek menguntungkan dari teh hijau, tidak hanya pada penyakit jantung tetapi juga pada diabetes tipe 2. **Metode:** Dalam penelitian ini, penyusunan ekstrak air teh hijau sudah terstandarisasi untuk (-) - epigallocatechin gallate (EGCG), komponen utama teh hijau. Efek ekstrak air teh hijau terhadap fluiditas darah dan diabetes dipelajari dalam 13 Fruktosa - Fed Rat (FFR). Tikus diberi diet tinggi fruktosa *ad libitum* selama satu minggu dan kemudian kombinasi dengan ekstrak air teh hijau setiap hari selama 6 hari. **Hasil:** Hasil penelitian menunjukkan, air ekstrak teh hijau dapat mengurangi 100  $\mu$ L darah dari wistar tikus secara signifikan ( $p < 0,01$ ) dengan alat *Micro-Channel Array Arus Analyzer (MC - FAN)*. Ekstrak air teh hijau juga memiliki efek yang kuat dalam mengurangi lemak perut ( $p < 0,05$ ), kadar glukosa darah ( $p < 0,01$ ) dan berat badan ( $p < 0,01$ ). **Diskusi:** Hasil ini menunjukkan bahwa ekstrak air teh hijau memiliki potensi menguntungkan untuk pengobatan diabetes dan mengurangi kekentalan darah.

Kata kunci : teh hijau , (-) - *epigallocatechin gallate*, HPLC, fluiditas darah , fruktosa-fed rat

### ABSTRACT

**Introduction:** Various studies have shown the beneficial effects of green tea, not only on cardiovascular diseases but also on type 2 diabetes. **Method:** In this study, the preparation of green tea water extract has been standardized to (-)-epigallocatechin gallate (EGCG), the major component of green tea. The role of green tea water extract on blood fluidity and diabetes diseases has been studied in 13 Fructose-Fed Rat (FFR). The rats were given high fructose diet *ad libitum* for one week and then combination with green tea water extract every day for 6 days. **Results:** The results show, green tea water extract can reduce 100  $\mu$ L blood passage times of wistar rat significantly ( $p < 0.01$ ) by *Micro-Channel Array Flow Analyzer (MC-FAN)* instrument. Green tea water extract also had strong effect in reducing abdominal fat ( $p < 0.05$ ), blood glucose level ( $p < 0.01$ ) and body weight ( $p < 0.01$ ). **Discussion:** These results suggest that green tea water extract may have beneficial effects for the treatment of diabetes and reduce blood viscosity.

**Keywords:** green tea, (-)-epigallocatechin gallate, HPLC, blood fluidity, fructose-fed rat

### INTRODUCTION

Tea has been consumed by many people in the world since ancient times. In various countries, green tea has been processed into a variety of foods and beverages such as cakes, ice cream and candy. Beside water, tea product also consumed in various types. At least there are four basic forms of tea product: green tea, oolong tea, pu'erh tea and black tea. These forms are different in process of production. Black tea was made from fermentation of tea leaf. The main component of green tea is (-)-epigallocatechin gallate (EGCG). During production process, oxidation of EGCG and another substance are promoted, so that most of these substances are oxidized. Because of that reason, level of EGCG and total catechin in tea were depend on type of tea. In

old leaf of green tea, EGCG content is higher than young leaf, and higher than oolong tea, and also higher than black tea and pu'erh tea (Lyn, et al, 2003).

There is good evidence from *in vitro* studies that green tea catechins have an important role in protection against degenerative diseases. EGCG from green tea extract has many activities such as anti-carcinogenic (Beltz et al, 2006, Spinella, et al, 2006), antioxidant (Luximon et al, 2006), anti-microbial activities (Paul, et al., 2006, Watson, et al., 2005) as well as anti-diabetic activity (Kim et al., 2013). Feeding of tea catechins has beneficial effect for the reduction of high fat diet induced obesity by increasing lipid metabolism. EGCG may modulate the distribution of Rstn protein, an adipocyte-specific

secretory hormone that can cause insulin resistance (Liu. et al, 2006). In the present study, the effect of standardized of green tea extract on EGCG as the active compounds, on blood fluidity, body weight and diabetes impact of fructose-fed Wistar rat were investigated. In addition, further investigations of the effect of this extract on some organs were also examined.

#### **MATERIALS AND METHODS**

Green tea was obtained from commercial product of PTPN 12, Indonesia. Heparin is included on Venoject tube 5 ml sterile from Kruuse. Fructose, casein, vitamins and minerals were purchase from Wako Pure Chemical Industry.

Male Wistar Rats were obtained from Clea Inc. Japan at 7 weeks of age. The fructose-fed diet mixed in Laboratory of Nutrition Physiology Kobe Women's University. The components of the fructose fed (100 g) were follows: 66% fructose, 22% casein, 12% lard plus essential vitamins and minerals. Samples experiment were given green tea extract 300 mg/kg body weight in water solution The control was made by given the same volume of distilled water. Wistar rat were kept in an animal room maintained at constant temperature ( $22 \pm 2^{\circ}\text{C}$ ). The rat consumed a fructose fed ad libitum and had free access to distilled water for 2 weeks. Body weight was measured every day. All procedures were performed in accordance with standards related to the care and management of experimental animals (including ethics) of the Kobe Women's University.

Wistar rat (8-10 weeks old) were fasted 12 h before the starting point of experiments. The rats were per orally administered with either a suspension of green tea powder on hot water at 9:20 a.m. Ten minute after the administration (at 9:30 a.m.), the rats were perorally administered with 1.0 ml of glucose solution containing 350 mg glucose. Blood glucose levels were measured before and 30, 60, and 120 min after the administration with glucose solution.

Mix 2.0 ml of fresh blood with 100  $\mu\text{l}$  heparin. Blood passage time was measured for 100  $\mu\text{l}$  of blood through an artificial capillary using MC-FAN (Micro-Channel Array Flow Analyzer). The Micro-Channel array consisting of 8,736 capillaries with 4.5  $\mu\text{m}$  in diameter and 30  $\mu\text{m}$  in length. The time needed was determined and expressed against 100  $\mu\text{l}$  saline solution adjusted to 12 seconds.

For statistical evaluation of the data in rat, repeated measures Student's t test were used. Difference of  $p < 0.05$  were considered significant. SPSS 14.0 for Windows software was used for all statistical analysis. Values in the text are means  $\pm$  SD.

#### **RESULTS**

In the chromatograms of the EGCG standard solution analyzed by HPLC, the peak of standard EGCG was identified as the single peak and separated completely from other compound in sample of green tea extract. Standard EGCG concentration has linier correlation ( $r = 0.9994$ ,  $p < 0.01$ ) with HPLC peak area. Concentration of EGCG in sample extract green tea was found  $4.53 \pm 0.03$  % and calculated from dry weight. This standardized sample was used for further examination in blood fluidity and diabetic impact of fructose fed rat.

Effect of green tea extract on blood parameter, fat and organs can be seen in table 1. Abdominal fat decreases significantly after drinking green tea. The average abdominal fat for the control group being 70% greater than in the animals with green tea. All of the blood parameter did not change significantly between two groups. Organs weight also has no significantly different except pancreas and kidney.

Effect of green tea water extract treatment on glucose metabolism in FFR was observed by oral glucose tolerance test (OGTT) at 2 week. Figure 2 shows that no significant differences of OGTT were observed between control and green tea extract groups.

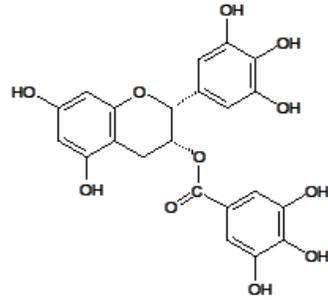


Figure 1. Structure of (-)-epigallocatechin gallate (EGCG) from green tea.

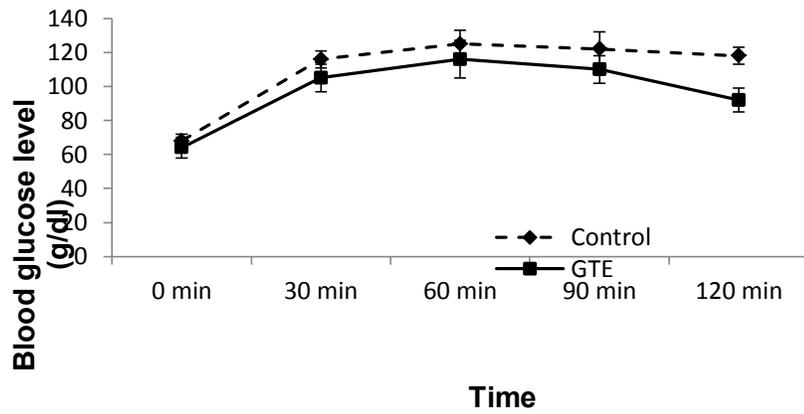


Figure 2. Oral glucose tolerance test of green tea extract (GTE) and control on Fructose-Fed Rats.

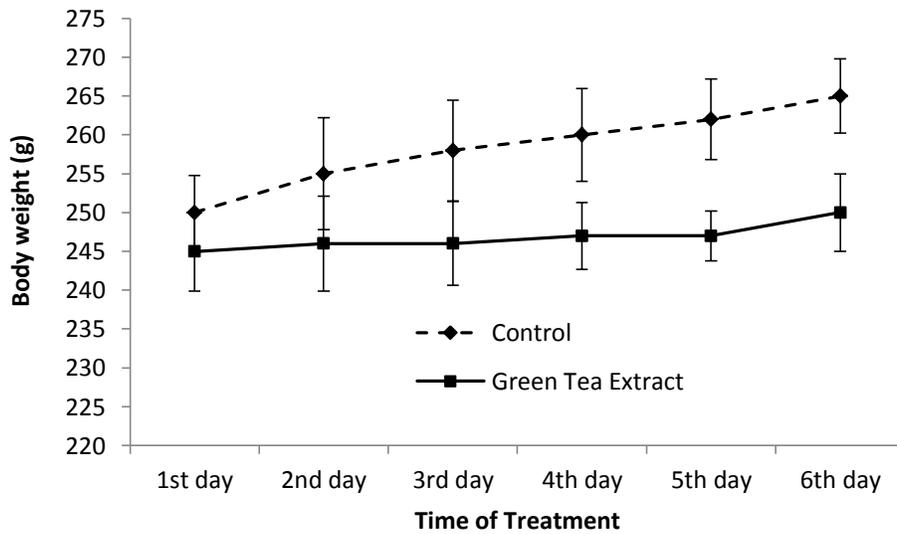


Figure 3. Green tea extract can reduces FFR body weight significantly for 6 days of treatment ( $p < 0.01$ ).

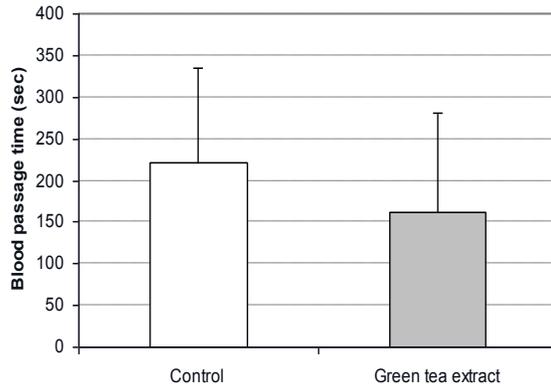


Figure 4. Effect of green tea extract on FFR blood fluidity measured by Micro-channel array flow analyzer (MC-FAN). Green tea extract lowers blood passage time significantly ( $p < 0.01$ ).

Tabel 1.

Effect of green tea extract on blood parameters, fats and organs weight of Fructose-Fed Rat (FFR)

	Control (n = 5)	Green Tea (n = 8)	P**
<i>Blood parameter</i>			
White blood cell ( $\times 10^2 / \mu\text{l}$ )	51.07 ± 14.25	54.04 ± 11.48	NS
Red blood cell ( $\times 10^4 / \mu\text{l}$ )	773.40 ± 15.75	792.42 ± 33.36	NS
Hemoglobin (g/dl)	15.21 ± 0.28	15.63 ± 0.46	NS
Hematocrite (%)	48.35 ± 0.85	49.35 ± 1.48	NS
MCV (fl)	62.53 ± 0.84	62.31 ± 0.96	NS
MCH (pg)	19.66 ± 0.24	19.73 ± 0.34	NS
MCHC (g/dl)	31.47 ± 0.20	31.69 ± 0.20	NS
Platelet ( $\times 10^4 / \mu\text{l}$ )	111.86 ± 8.64	113.28 ± 15.80	NS
<i>Fat</i>			
Abdominal fat (g)	3.58 ± 0.68	2.03 ± 0.46	S
Abdominal fat (g/100 g b.w)	1.48 ± 0.23	0.87 ± 0.20	S
<i>Organs</i>			
Spleen (g/100 g b.w)	0.21 ± 0.02	0.20 ± 0.04	NS
Pancreas (g/100 g b.w)	0.20 ± 0.09	0.27 ± 0.05	S
Kidney (g/100 g b.w)	0.42 ± 0.02	0.35 ± 0.05	S
Adrenal (g/100 g b.w)	0.0073 ± 0.0034	0.0644 ± 0.1390	NS
Genital (g/100 g b.w)	0.63 ± 0.07	0.69 ± 0.05	NS
Lung (g/100 g b.w)	0.45 ± 0.04	0.48 ± 0.04	NS
Thymus (g/100 g b.w)	0.12 ± 0.08	0.10 ± 0.02	NS
Liver (g/100 g b.w)	3.88 ± 0.17	3.58 ± 0.31	NS
Heart (g/100 g b.w)	0.33 ± 0.01	0.35 ± 0.03	NS

\*Results are expressed as means ± SD.

\*\*Significance of difference between control and green tea ( $p < 0.05$ ); S=Significantly; NS=Not Significantly ( $p > 0.05$ )

## DISCUSSION

The oral glucose tolerance test (OGTT) measures the body's ability to use glucose, as an energy source for the body. An OGTT can be used to diagnose prediabetes and diabetes. But, there is no significant differences were observed between control and green tea extract groups, although some

researcher expects that high-fructose feeding could induce insulin resistance (Nakagawa et al., 2002). This fact may be due to the treatment is too short so insulin tolerance has not happened.

Green tea extract has been known as chemo preventive agent in various diseases, and EGCG as its major component has important role for this

activity. EGCG contain in some green tea leaf and products are different. Many *in vitro* studies on green tea report mechanisms consistent with protection against degenerative diseases (Huang et al., 1999, Nakagawa et al., 2002). Nevertheless, many of these studies used various concentrations of catechin and thus do not reflect certain catechin concentrations found in herbal extract. It is difficult to decide these results to EGCG concentration. However, because of the lack information of active compounds role and also biological effects of the conjugates, thus, animal studies are more relevant for investigating the physiological effects of catechins. This research studies the most interesting *in vivo* animal of the biological effects of standardized green tea extract on EGCG before used as herbal medicine to get sufficient effects.

The results showed that consumption of green tea extract for 6 days caused a suppressive effect on weight gain and visceral fat accumulation in FFR. Green tea extract contains caffeine and EGCG as the principal constituents, and these constituents showed a tendency to suppress body weight gain and visceral fat accumulation. Studies conducted with human subjects report reduced body weight and body fat, as well as increased fat oxidation and thermogenesis (Wolfram et al., 2006). Thus, these constituents (EGCG and coffein) are suggested to be partially involved in the suppressive effect on body weight gain and visceral fat accumulation.

Blood rheology is now receiving increasing attention as an important potential contributory factor to diabetic angiopathy (Le Dévéhat et al., 2004). Therefore, monitoring of the blood fluidity becomes very important in handling of diabetics impact. The use of green tea extract may increase blood fluidity, thereby reducing the impact of diabetic angiopathy.

## CONCLUSIONS AND RECOMENDATION

### Conclusions

It can be concluded that the water extract of green tea can decrease blood passage time of FFR, suppress body weight and visceral fat accumulation, but have no effect on blood parameter, insulin tolerance and organs weight except kidney and pancreas.

### Recommendation

According to the result, it is suggest that green tea water extract may has beneficial effects for the treatment of diabetes and reduce blood viscosity.

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