

RESEARCH ARTICLE

The Effect of Green Tea and Stevia Extract on Liver Histopathology Wistar Rats Type II Diabetes Mellitus

Elmawati Novit¹, Udadi Sadhana², Vega Karlowee², Endang Mahati^{3*}

¹ Medical Student, Faculty of Medicine, Diponegoro University, Semarang

² Departement of Pathology Anatomy, Faculty of Medicine, Diponegoro University, Semarang

³ Departement of Pharmacology, Faculty of Medicine, Diponegoro University, Semarang

*corresponding author, email: endang.mahati@fk.undip.ac.id

ABSTRACT

Introduction: Glucose metabolism abnormality on type 2 diabetes mellitus (T2DM) caused liver damage due to dyslipidemia and increasing oxidative stress. Antidiabetic and antioxidants within green tea leaf and stevia leaf extract prevent liver damage due to T2DM complications. **Objective:** To investigate green tea and stevia leaf extract effects on liver histopathology on T2DM rats.

Methods: This study used Post Test Only Control Group Design. A total of 30 Wistar rats were divided into 5 groups: control group (C-G), normal rats; negative control group (NC-G), T2DM induction; positive control group (PC-G), treated by Metformin; the first treatment group (T1-G), was treated by green tea leaf extract and stevia leaf extract; and the second treatment group (T2-G) was treated by a Metformin also green tea and stevia leaf extract. The treatment was given to subjects for 21 days starting 3 days after the induction of Nicotinamide (NA) and Streptozotocine (STZ). Histopathological assessment of liver cells uses assessment parameters in the form of features of necrosis, vacuolization, sinusoidal dilation and number of inflammatory cells. Hypothesis will be tested by Kruskal-Wallis ($p < 0,05$) dan Mann-Whitney test ($P < 0,05$)

Results: The difference of liver damage in all group shows a significant difference, $p < 0.05$. Significant differences in liver damage scores between the 2 groups there are between the negative control group and the control group (0.031), positive control (0.042), and second treatment group (0.011), and between first treatment group and second treatment group (0.041).

Conclusion: The administration of green tea extract, stevia leaf, and standard drug may reduce liver damage score of Wistar with T2DM

Keywords : green tea leaf, stevia leaf, liver, T2DM

ABSTRAK

Pendahuluan: Abnormalitas metabolisme glukosa pada diabetes mellitus tipe 2 (DMT2) dapat menyebabkan kerusakan hepar akibat dislipidemia serta peningkatan stres oksidatif. Kandungan antidiabetes dan antioksidan pada ekstrak daun teh hijau serta daun stevia dapat mencegah kerusakan hepar akibat komplikasi DMT2. **Tujuan:** Untuk mengetahui efek ekstrak daun teh hijau dan daun stevia terhadap gambaran histopatologi hepar akibat komplikasi DMT2.

Metode: Penelitian ini menggunakan rancangan *Post test Only Control Group Desain*. Sebanyak 30 tikus Wistar dibagi menjadi 5 kelompok: kelompok control (C-G), tikus normal; kelompok kontrol negatif (NC-G), induksi DMT2; kelompok kontrol positif (PC-G), diberi Metformin; kelompok perlakuan pertama (T1-G), diberi ekstrak daun teh hijau dan daun stevia; dan kelompok perlakuan kedua (T2-G), diberi Metformin serta ekstrak daun teh hijau dan daun stevia. Perlakuan diberikan selama 21 hari dimulai 3 hari setelah induksi Nikotinamide (NA) dan Streptozotocin (STZ). Penilaian histopatologi sel hepar menggunakan parameter penilaian berupa gambaran nekrosis, vakuolisasi, pelebaran sinusoid dan jumlah sel radang. Uji hipotesis yang dilakukan adalah uji *Kruskal-Wallis* ($p < 0,05$) dan uji *Mann-Whitney* ($P < 0,05$).

Hasil: Perbedaan kerusakan hati pada semua kelompok menunjukkan perbedaan yang signifikan dengan nilai $p = 0,023$. Perbedaan bermakna skor kerusakan hepar antara 2 kelompok yaitu antara kelompok kontrol negatif dengan kelompok kontrol (0,031), kontrol positif (0,042), dan kelompok perlakuan kedua (0,011), serta antara kelompok perlakuan pertama dan kelompok perlakuan kedua (0,041).

Kesimpulan: Pemberian kombinasi ekstrak daun teh hijau dan daun stevia serta obat standar dapat menurunkan skor kerusakan hepar tikus Wistar DMT2.

Kata Kunci: daun teh hijau, daun stevia, hepar, DMT2

INTRODUCTION

Diabetes mellitus (DM) is a non-communicable disease that ranks fourth (4%) as a cause of death in the world, which killed 1.6 billion people in the world in 2016 (World Health Organization, 2018). Based

on the data on Riskesdas (2018) there has been an increase in the prevalence of DM sufferers from 2013 to 2018. The continuous increase in prevalence in DM will certainly add to the country's burden in terms of the health economy, moreover, health problems in

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Indonesia are double burden (Effendi and Waspadji, 2012). From several diabetic classifications, almost 90% of patients experience type 2 DM (T2DM) (World Health Organization, 2016)(Sherwood, 2014). Type 2 diabetes mellitus as one of the most common metabolic diseases can cause various complications and abnormalities in the liver. Green tea which is rich in antioxidants, (Hidayat and Napitupulu, 2015) and stevia which is known as anti-hyperglycemic and antioxidant can help inhibit organ damage caused by T2DM (Assaei et al., 2016). However, its effect on liver damage due to T2DM remains unclear.

Type II diabetes mellitus can cause various complications, one of which is fatal is the liver complication (Mohamed et al., 2016). Abnormalities of insulin secretion and insulin resistance in T2DM can cause complications in the liver such as chronic liver disease (CLD), cirrhosis, and abnormalities of liver fat metabolism that develop into fatty liver (Sulaiman et al., 2012)(Bilous and Donnelly, 2014)(Mohamed et al., 2016). In T2DM there is also an increase in oxidative stress which can cause inflammation of the liver, so that it is at risk for fibrosis, cirrhosis, and ultimately resulting in hepatocellular carcinoma (Mohamed et al., 2016).

Green tea has antioxidant content in the form of quercetin which is higher than black tea. In addition to high quercetin, green tea catechins have antioxidant activity 100 times more effective than vitamin C and 25 times more effective than vitamin E. Antioxidants are very important for the body, because antioxidants help the enzyme superoxide dismutase (SOD) to get rid of free radicals that are harmful to the body (Hidayat and Napitupulu, 2015). In addition, stevia is a sweetener and sugar substitute extracted from the leaves of the *Stevia rebaudiana* plant. Stevia contains non-calorie natural sweetener and is able to produce sweet taste 250-300 times sweeter than cane sugar (Khiraoui et al., 2017). Stevia leaf extract can increase insulin levels and provide anti-hyperglycemic effects through PPAR γ mechanism and has antioxidant properties (Assaei et al., 2016).

Aim of this study, is to evaluate directly the effect of green tea leaf extract and stevia leaf on the liver histopathology of Wistar rats T2DM. Thus this research can be a scientific basis and provide information about the benefits of green tea extract and stevia leaf extract on liver organs in T2DM.

METHODS

This study uses a true experimental laboratory design with posttest only control group design. The

samples in this study were male Wistar rats with an average body weight of 200-220 grams, aged 2-3 months, rats in a healthy and active state, there were no anatomical abnormalities and rats did not die before the termination period. A total of 30 Wistar rats were adapted for 1 week and then random allocation sampling was carried out and divided into 5 groups and each group consisted of 6 rats. Five groups consisted of a control group (C-G), Wistar rats were fed and drank ad libitum as a standard; negative control group (NC-G), Wistar rats induced T2DM only; positive control group (PC-G), Wistar rats after inducing T2DM were given a standard drug namely Metformin 150 mg/kg/day orally; the first treatment group (T1-G), Wistar rats after T2DM induction were given green tea extract 600 mg/kg body weight and stevia leaves 300 mg/kg body weight orally using oral gauge; the second treatment group (T2-G), Wistar rats after T2DM induction were given green tea extract 600 mg/kg body weight and stevia leaves 300 mg/kg body weight orally and standard drug Metformin 150 mg/kg/day was given orally. The administration of green tea extract and stevia leaves was done for 21 days starting 3 days after the induction of T2DM. This study was approved by the Research Ethics Commission (KEPK) of the Faculty of Medicine, Diponegoro University/RSUP Dr. Kariadi Semarang for all animal experiments with number of ethical clearance 47/EC/H/KEPK/FK-UNDIP/V/2019.

Induction of T2DM rats

Streptozotocine (STZ) was injected intraperitoneally by 45 mg/kg body weight 15 minutes after intraperitoneal niacinamide (NA) administration of 110 mg/kg. NA induction followed by STZ induction can provide T2DM effects in experimental animals, this is because NA has antioxidant capacity that can reduce the cytotoxic effect of STZ on pancreatic β cells (Ghasemi, Khalifi and Jedi, 2014).

Preparation of green tea extract

Blend the dried leaves of green tea to powder. A total of 250 grams of green tea extract is made by multilevel maceration and mixed with 70% ethanol as a solvent in the ratio of 1:10 times the simplicity of 2000 ml. The extraction results are dried using a rotary evaporator. Green tea leaf extract is mixed with 1 ml of water and given through oral gauge (Kurnia et al., 2015).

Preparation of stevia leaf extract

Stevia dried leaves mashed with a blender. Two

Table 1. Wistar rats liver cell damage score

Variables	Groups					P value (Kruskal Wallis)
	C-G	NC-G	PC-G	T1-G	T2-G	
	N=5 X(+SD)	N=5 X(+SD)	N=5 X(+SD)	N=5 X(+SD)	N=5 X(+SD)	
Total score of damage	0.60 (± 0.548)	1.60 (± 0.548)	0.80 (± 0.447)	1.40 (± 0.894)	0.20 (± 0.447)	0.023

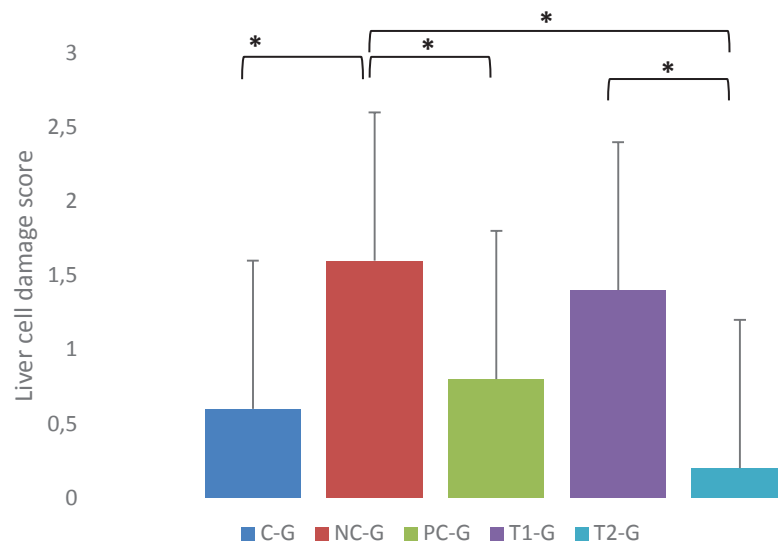


Figure 1. The histogram of differences between groups.

*The difference test analysis with Man Whitney is significant if $p < 0,05$

hundred grams of stevia powder was extracted stratified by the maceration method in a 70% ethanol solution with a total volume of 2400 ml. The extraction results are concentrated with a rotary evaporator. The extract was dissolved in 1 ml of water and then given orally using an oral gauge (Iman et al., 2017).

Production and assessment of liver histopathology preparations

All rats were dissected on the 31st day for organ harvesting and histopathological preparation. The liver is fixed in 10% normal buffer formalin (BFN) for a minimum of 12 hours, then tissue management is carried out according to the standard method of making histopathological sedans and stained with routine painting of Hematoxylin Eosin (HE). The preparation is analyzed under a light microscope. The parameters of liver damage assessed were necrosis, vacuolization, sinusoidal dilation and number of inflammatory cells. Assessments were carried out with parameters in the form of necrosis (score 0, no necrosis; 1, there was necrosis), vacuolization (score 0, no vacuolization; 1, there was vacuolization), sinusoid dilation (score 0, no sinusoid widening; 1, there was widening sinusoid), as well as the number of inflammatory cells (0, no inflammatory cells; 1, mild; 2, moderate; 3, severe;

4, very severe). After calculating every parameter of liver damage, the total is calculated and the result is considered a Wistar rats liver cell damage score.

Statistic analysis

All data are tested with the Shapiro-Wilk and Levene test to determine whether the data distribution is normal and homogeneous. The results of statistical analysis showed that the data had an abnormal distribution ($p < 0.05$). Therefore to determine differences in liver cell damage scores between parameters tested using a non-parametric test by Kruskal walls, followed by the Mann-Whitney Post Hoc test. Statistical analysis was considered as significant when $p < 0.05$.

RESULTS

After oral administration of green tea and stevia leaf extracts for 21 days in the two treatment groups, the results are shown in Table 1 below.

The highest average liver damage score is in the negative control group, followed by the first treatment group, the positive control group, the control group, and the lowest average liver damage score is in the second treatment group.

Kruskal-Wallis statistical analysis obtained $p = 0.023$, which means there are significant differences.

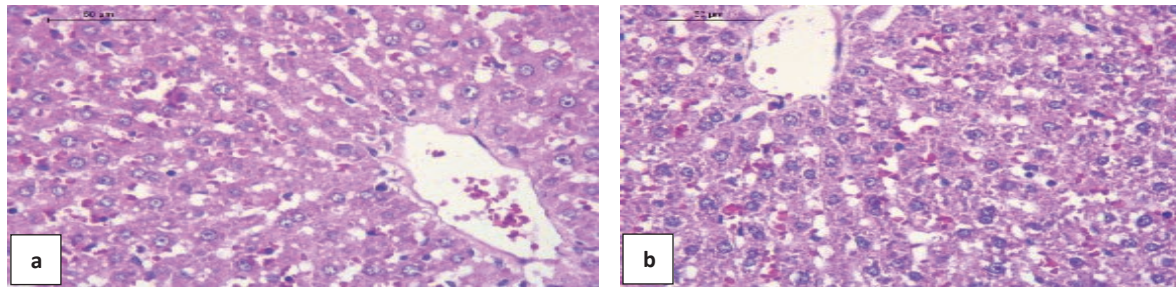


Figure 2. Histopathology of Liver Wistar rat 400x by Hematoksilin Eosin (HE). Comparison between histopathological features in control group (a) and second treatment group (b). Seem histopathological features in second treatment group has similar features compared to control group.

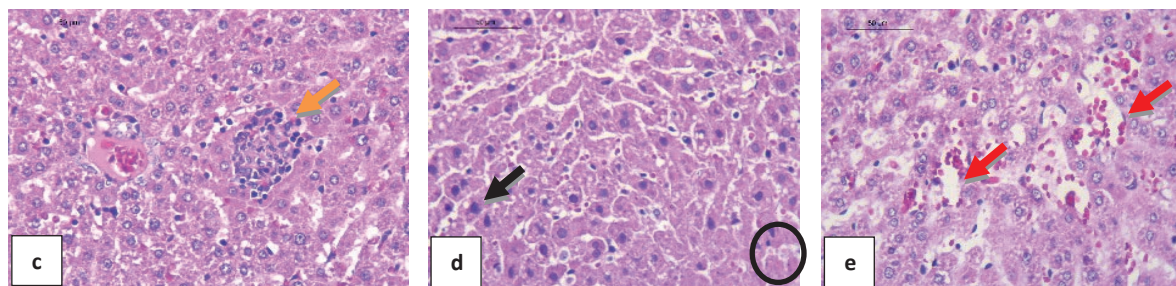


Figure 3. Histopathology of Liver Wistar rat 400x by Hematoksilin Eosin (HE). Liver damage on negative control group, shows necrosis (black circle) and vacuolization (black arrow) (d), sinusoidal dilation (red arrow) (e), and inflammatory cell (green arrow) (c).

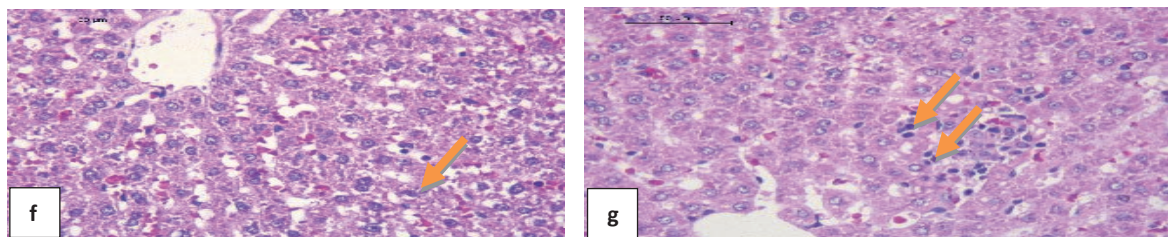


Figure 4. Histopathology of Liver Wistar rat 400x by Hematoksilin Eosin (HE). Comparison between histopathological features positif control group (f), with first treatment group (g). First treatment group seems more inflammatory cells (green arrow) then positif control group.

The next test was conducted by the Man-Whitney test which found that there were significant differences between the two groups between the control and negative control groups, the negative control group and the positive control group, the negative control group and the second treatment group, and between the first treatment group and second treatment group. The difference is shown by the value of $p < 0.05$.

DISCUSSIONS

The results showed there were significant differences in the score of liver cell damage due to complications of T2DM between two or more groups with a value of $p = 0.023$. From this study, the results obtained in rats first treatment group (T1-G) found no significant difference with the group negative control (NC-G), showed that the administration of green tea

leaf extract and stevia leaf did not affect repairing liver cell damage due to complications of T2DM. Although there was no significant difference between liver damage scores in first treatment group and negative control group, the administration of green tea extract and stevia leaf in this study was able to reduce blood glucose levels when in accordance with the results of statistical processing which showed a significant decrease in blood sugar with a probability value of $p = 0.043$ ($p < 0.005$).

There was no significant difference between the first treatment group and the negative control group not in accordance with previous studies which stated that green tea with a dose of 600 mg/kg body weight can reduce blood glucose in diabetic rats and improve liver damage in rats, and intake of stevia extract resulted in a significant decrease ($P < 0.05$) blood glucose levels and

a significant increase in insulin levels (17.82 μ IU/mL) and liver glycogen levels in diabetic rats, compared with diabetic and non-diabetic control rats after 8 weeks of the study period (Ilma, 2016)(Bayat et al., 2017).

Theoretically, green tea leaf extract and stevia leaf can be used as an antidiabetic. Green tea itself acts as an antidiabetic because one of the components of the catechin, Epigallocatechin Gallate (EGCG), is known to inhibit intestinal glucose uptake by glucose-dependent glucose transporters namely SGLT1 transporters. Besides, EGCG mimics insulin, increases tyrosine phosphorylation of insulin receptors and insulin receptor substrates, and reduces gene expression of the gluconeogenic enzyme phosphoenolpyruvate carboxykinase. In pancreatic β cells, EGCG can also repair these cell damage due to induction of cytokines in vitro and prevent decreases in island mass caused by treatment with several low doses of STZ in vivo (Chacko et al., 2010). While stevia can act as antidiabetic because content of natural non-calorie sweet natural molecules of steviol glycosides (SGs) containing rebaudioside A and aglycon steviol. These substances have the potential to activate Ca^{2+} activated cation channels and perception of sweet taste and increase glucose secretion induced by Transient receptor potential cation channel subfamily M member 5 (TRPM5) glucose expressed in type II taste receptor cells and pancreatic β cells (Pandey, 2018). Additionally stevia leaf extract contains several biomolecules that increase biomolecules that increase insulin receptor sensitivity, stimulates pancreatic β cells to release insulin and increase insulin content in INS-1 cells, by inducing genes involved in glycolysis (Raini and Isnawati, 2011). In this study there was a decrease in blood sugar when it showed that tea leaf extract green and stevia leaves can be used as antidiabetic so according to the theory above, but not enough to protect liver damage due to complications of T2DM.

Other results from this study indicate that there are significant differences between the first treatment group with the second treatment group. This is evidenced by the probability value $p < 0.05$ with the liver damage score in the first treatment group higher than the second treatment group. This shows that the addition of green tea leaves and stevia leaves can reduce liver score damage when combined with standard drugs in this study is metformin.

Theoretically, metformin is a T2DM drug that can reduce blood sugar levels and reduce free fatty acids in the blood. In addition to working in the blood, metformin works directly in the liver to prevent lipolysis and fat oxidation in the liver. In addition,

Metformin can protect hepatocytes from cell death due to saturated fatty acids (Liu et al., 2013). The existence of direct protection against the liver is what allows the administration of metformin alone is able to repair liver cell damage due to T2DM. This is in line with the results of the study which showed a significant difference between the negative and positive control groups.

According to the theory of green tea and stevia leaf has activity as a hepatoprotection, this is related to the antioxidant content in the extract of green tea leaves and stevia leaves. Antioxidants in green tea leaf extract and stevia leaf can prevent liver damage due to free radicals resulting from metabolic abnormalities in T2DM. Increased free radicals can cause oxidative stress in tissues including liver tissue (Shen et al., 2017). Free radical attack on liver cells can cause damage to the mitochondria and cytoplasmic membrane which causes the process of apoptosis (Mohamed et al., 2016). In addition, free radicals can interfere with lipids, induce necrosis, strengthen the inflammatory response and cause a number of changes in DNA such as DNA division and DNA-protein cross-linking which ultimately cause DNA mutations and increased ROS production. Reactive oxygen species (ROS) themselves play an important role in producing liver damage and initiating liver fibrogenesis by stimulating the production of profibrogenic mediators from Kupffer cells and circulation of inflammatory cells and directly activating hepatic stellate cells (Uribe, 2012). For neutralizing these toxic properties requires enzymatic reactions and antioxidants from outside the body, one of which is contained in green tea extracts and stevia leaves. Green tea extract is known to contain antioxidants such as flavonoids and catechins which have free radical scavenger activity higher than antioxidants in vitamin C and vitamin E. While stevia leaf extract has antioxidant type phenols which have phenol content reaching 91 mg/g stevia leaves, the phenol has a greater ability to overcome free radicals and prevent lipid peroxidation than other antioxidants such as hydroxytoluene butylation, hydroxyacetyl butylation, and tertiary butyl hydroxyquinone. In the end, the combination of antioxidants is able to prevent apoptosis of liver cells to further prevent liver damage due to T2DM conditions (Mohd-radzman et al., 2013)(Liu et al., 2013).

Giving green tea extract and stevia leaf in first treatment group, although it did not show significantly different results than the negative group, it still had a better impact on the liver. This can be seen from the data in table 1 which shows the average liver damage score is lower in the second treatment group than the positive

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control group. The data shows that the administration of green tea extract and stevia leaves can increase liver protection better with standard drugs than just using standard drugs.

CONCLUSIONS

Giving a combination of green tea extract and stevia leaf as well as standard drugs can reduce the histopathological damage score of the liver of Wistar rats in T2DM compared to standard drug users only. In this study T2DM induction was carried out by injection of Streptozotocine which has a direct impact on the liver so the liver damage can be bias between T2DM complications and toxic effect of STZ. Therefore, in subsequent liver organ studies, other method of T2DM induction can be used which are not toxic to the liver. In addition, graded doses of green tea and stevia leaves extract can be applied.

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CONFLICT OF INTEREST

No conflict of interest has been declared by the author.

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