Green Mustard Ethanol Extract (Brassica Rapa L.) Leaf Can Cell Damage (8-Hydroxy-2-Dioxiguanosine) In The Wistar Rat Hyperglicemic

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Abstract. Hyperglicemia is a condition in which glucose levels exceed normal limits. The condition of chronies hyperglycemia result in increased free radical production resulting in oxidative stress. Oxidative stress is one component in the mechanism of damage in the pancreas of hyperglycemia Wistar rat. This Research true experimental with The Randomized Pre and Posttest Control Group Design. Oxidative stress is demonstrated by increasing 8-hydroxy-2-dihidroxiguanosin (8-OHdG) levels. Antioxidant compound active phenol in brassica rapa L. Phenol is group contained in medical plants can reduce the occurrence of hyperglycemia. This study to determine the effect of giving brassica rapa L ethanol extract (Brassica rapa L) in decreasing reducing 8-OHdG levels in hyperglycemics Wistar rats. Wistar rats that have experienced hyperglycemia were divided into 6 groups. Po (Normal groups), P1 (positive control with given glibenclamide), and P3, P4, P5 (treatment was given orally with green mustard of 10 mg/ kg BW; 15 mg/kg BW ; 20mg/kg BW) can decreasing 8-OHdG (8-Hydroxy-2-dioxiguanosin). The result of treatment ethanol extract brassica rapa L showed P0: 2.440 ng/dL. P1:7.482 ng/dL; P2 :2.772 ng/dL; P3:5.158 ng/dL; P4: 2.304 ng/dL. The content of phenol groups in capturing of neutralizing the radical scavenging in hyperglycemic Wistar rats. One Way Anova analysis and Post Hoc Study showed that ethanol extract brassica rapa L extract doses 5.0 mg/kgBW was able to significantly (P≤ 0.05) can decreasing damage cell in hyperglycemic Wistar rats.

1. Introduction
Hyperglycemia is a condition in which glucose levels in blood plasma exceed normal limits. Fasting blood glucose levels of patients have increased above 110 mg / dl and blood glucose 2pp (post prandial above 140 mg / dl [1]. In The world Since 2000, diabetes sufferers in Indonesia have experienced an increase, the World Health Organization (WHO) predicts that in 2030 people with diabetes will reach 21.3 million people. Based on the latest epidemiological studies, Indonesia has entered into a type 2 DM epidemic. Nearly 80% of the prevalence of diabetes mellitus is caused by lifestyle. The lifestyle of the world community, especially Indonesia in the last few years, shows a change in eating patterns from traditional and nutritious food to fast food that is low in nutrition (junk food).

The process of auto-oxidation in hyperglycemia and glycation reactions results in electron release. The release of these electrons triggers the formation of free radicals. Increased production of free radicals results in oxidative stress [2]. Oxidative stress is an event where free radicals in the form of reactive molecules arise through a biochemical reaction from normal cells that damage cell membranes and cause various bodily functions. Oxidative stress is one component of the mechanism of tissue damage in humans. Therefore, the condition of hyperglycemia will increase oxidative stress, and oxidative stress will worsen the health condition of the sufferers, so that antihipercglycemia is needed.
Based on the results of Ref. [3], this green mustard positively contains alkaloids, terpenes, tannins, saponins, and glycosides. Alkaloids are proven to have the ability to regenerate damaged pancreatic β cells. Terpenes function as antidiabetic because terpenes are the main components of essential oils while saponins function to increase glucose homeostasis by increasing insulin sensitivity. Corrugated mustard greens contain biologically active compounds such as flavonoids including isorhamnetin, kaempferol and quercetin glycosides, phenyl propanoid derivatives, indole alkaloids, and sterol glucosides. The use of mustard greens as an anti-diabetes drug is still rarely found. Generally people use mustard greens to make vegetables.

There has been no scientific study on the effect of ethanol extract of mustard greens, on decreasing blood glucose levels and reducing oxidative stress conditions which are characterized by decreasing levels of 8-OHdG (Brassica rapa L.) which has the potential to be anti-hyperglycemia. (Flowering plants); Super Divisions: Angiosperms (Producing seeds); Class: Dicotyledoneae (two pieces / dicot); Order: Brassicales; Family: Brassicaceae; Genus: Brassica; Brassica rapa L. species.

Green mustard, is one of the leaf vegetables that is still a family with cabbage-cropped, cabbage-flowered, broccoli and turnips derived from the brassicaceae family which has high economic value. Stems of mustard greens usually have a height of 30-160 cm, unbranched and taproot. The leaves have varying shapes and widths, finely pinnate leaf bones pale green and bitter taste. This plant is a type of vegetable that is widely used by people from various groups. Based on research on the phytochemical content of green mustard leaf, it has alkaloids, terpenes, tannins, aponins, and glycosides [3].

Diabetes is a type of disease that has potential complications (causing other diseases). Diabetes is associated with an increase in blood sugar levels, and cause damage to blood vessels, nerves and other internal structures. Diabetes mellitus is a metabolic disease characterized by hyperglycemia that occurs due to abnormal insulin secretion, insulin action or both. Chronic hyperglycemia in diabetes causes long-term damage, dysfunction or failure of several body organs, especially the eyes, nerves, heart and blood vessels. The mechanism of type 1 diabetes is based on the lack of insulin production, whereas the mechanism of diabetes 2 is based on the body's insensitivity to insulin. The World Health Organization (WHO) estimates that 177 million of the world population has diabetes, this number will continue to grow to exceed 300 million by 2025. In 2000 Indonesia ranks fourth in the ranks of countries with the highest number of people with diabetes mellitus in the world after India (31.7 million people), China (20.8 million) and the United States (17.7 million people). This figure is expected to increase to 21.3 million people in 2030 diabetics. Diabetes mellitus based on etiological classification can be divided into four, namely: type 1 diabetes mellitus (insulin dependent diabetes), diabetes mellitus type 2 (diabetes that is not dependent on insulin), gestational diabetes mellitus and other specific types of diabetes mellitus. Type 1 diabetes mellitus is more common at a young age, but can also occur in adulthood. This disease is caused by a lack of insulin production followed by high glucagon plasma which is triggered by pancreatic β cell failure. More than 95% type 1 diabetes mellitus is caused by an autoimmune process and 5% due to idiopathic pancreatic β cell destruction [4].

This happens because of insulin resistance, which is a condition where the body fails to use adequate insulin or is unable to use insulin properly, and is coupled with relative insulin deficiency. Insulin deficiency is a relative condition of the body producing insulin, but it is not enough to convert food or glucose into energy due to obesity. The two main disorders that cause type 2 diabetes mellitus are impaired insulin secretion and insulin resistance. The main disorder experienced by people with type 2 diabetes mellitus begins at the level of pancreatic β-cells with manifestations of impaired insulin secretion [5]. Oxidative stress is a condition in which the amount of free radicals in the body exceeds the body's capacity to neutralize it. As a result the intensity of the oxidation process of normal body cells becomes higher and causes more damage. Increased glycosidation and liposidation results in plasma and protein tissues due to increased oxidative stress in diabetes mellitus.

Various mechanisms have been proposed for the contribution of hyperglycemia as a result of oxidative stress, one of which is the possibility of glucose oxidation as a source of ROS. Streptosototozine administration is a fast way to produce experimental diabetic (hyperglycemic) conditions in experimental animals. Streptosototozine can be used intravenously, intraperitoneally, intramuscularly and subcutaneously. Intravenous injection is the administration of drugs by inserting drug fluids directly into veins, so that drugs directly enter the body through blood circulation. Intraperitoneal is the
administration of drugs in the peritoneal cavity, around the abdomen or abdomen. Subcutaneous is the provision of drugs to be released for longer, injecting under the skin and intended as a reservoir. Based on the results of Ref. [6], diabetic rats induced by alloxan 150 mg/kgBB intraperitoneally (ip), on the second day after streptosotozine induction, their blood glucose levels had increased.

The mechanism of action in causing selective destruction of pancreatic beta cells is not yet clearly known, however the damage occurs in an essential substance in the pancreatic beta cells causing the reduction of insulin-carrying granules in the pancreatic beta cells. Aloxan increases insulin and protein release from pancreatic beta cells. Streptosotozine might exert diabetogenic effects by damage to beta cell membranes by increasing permeability. The action of streptosotozine is mediated by free radicals.

The toxic action of streptosotin on beta cells is initiated by free radicals formed by redox reactions. Radical anion which is converted into ketoaldehyde and superoxide anion radicals.

Research on the mechanism of action of streptozotosin in vitro shows that streptosotozine induces the release of calcium ions from mitochondria which results in the process of cell oxidation being disrupted. The release of calcium ions from the mitochondria results in homeostasis which is the beginning of cell death.

Eight Hydroxy-2-Dioxiguonosin (8-OHdG) is a modified nucleic base, generally 8-OHdG is known as a by-product of DNA damage that is secreted in serum DNA repair. In DNA, guanine nucleotides are the most sensitive nucleotides in the presence of free radicals, so that oxidation will easily occur where the oxidation of guanine nucleotides will form 8-OHdG. Association of Reactive Oxygen Species (ROS) and the use of 8-OHdG as a biomaker of oxidative stress and cell damage [5]. Generation 2 with greater hypoglycemic potential includes gliburid (glibenclamide), glipizide, glycazzide and glimepiride. Gliburid (glibenclamide) has 200x more potency than talbutamide, its half-life is about 4 hours, its metabolism is in the liver, in a single dose only 25% of its metabolism is excreted in urine, the rest through bile.

Glibenclamide is a hypoglycemic oral sulfonyl urea derivative that works actively to reduce blood sugar levels. The mechanism of action of this class of drugs is often called insulin secretagogues, it works to stimulate insulin secretion from the granules of β-langehans pancreatic cells glibenclamide works by stimulating insulin secretion from the pancreas. Therefore glibenclamide is only useful in adult diabetics whose pancreas is still capable of producing insulin. In oral use glibenclamide is partially absorbed rapidly and spread throughout the extracellular fluid, mostly bound to plasma protein [8].

A single dose application of glibenclamide will reduce blood sugar levels within 3 hours and this level can last for 15 hours. Glibenclamide is excreted with faeces and as metabolites with urine Glibenclamide dose 5mg/day, single dose maximum of 10 mg (Indonesian Pharmacists Association (IAI), 2010). The time to reach the maximum concentration in blood (T max) of glibenclamide is 3 hours [5].

2. Materials and Method
The place of research was conducted at the Research Laboratory of the Department of Chemistry, Faculty of Mathematics and Natural Sciences, Udayana University. Processing of samples and laboratory of natural materials at the Faculty of Pharmacy for the maintenance of Wistar rats and at Udayana University's Analytical UPT Laboratory for purification.

The material used was Green mustard (Brassica rapa L.) obtained from Sumber Village, Sanankulon District, Denpasar Bali Regency. Glibenclamide as a comparison drug for diabetes drugs and alloxan monohydrate as an inducer of diabetes, 70% ethanol, FeCl3, Mg powder, concentrated HCl, 1% HCl, Mayer reagents, anhydrous acetic acid, concentrated sulfuric acid, chloroform, rat Wistar, feed standard, streptozotosin, EDTA, TCA 15 % , TBA solution 0,37% in the HCL 0,25N, The material used was Green mustard (Brassica rapa L.) obtained from Sumber Village, Denpasar Bali. Glibenclamide as a comparison drug for diabetes drugs and alloxan monohydrate as an inducer of diabetes, 70% ethanol, FeCl3, Mg powder, concentrated HCl, 1% HCl, Mayer reagents, anhydrous acetic acid, concentrated sulfuric acid, chloroform, male Wistar rats, standard feed, HCl alloxan, EDTA solution, TCA and aquadest.

The equipment used in this study include: UV-VIS spectrophotometer, GC-MS, spatula, blender, glass jar, glassware, funnel, rotary vacuum evaporator, analytical balance, µpipet 100 µL, water-bath,
centrifuge, test tube centrifugation), spuite, EDTA tubes, filter paper, alluminium foil, dripping pipettes, sonde devices, mouse cages, gloves and mask. Some stages of sample preparation include: fresh samples of Green mustard (Brassica rapa L.), washed using clean water and drained. Then dried by aerated, without exposure to sunlight. Samples of Green mustard (Brassica rapa L.) which has been dried then mashed using a blender and weighed with an analytical balance. 1000 g of Mustard Green (Brassica rapa L.) powder was extracted by maceration using 96% ethanol solvent until all the powder was submerged in the solvent. Immersion is carried out for ± 48 hours repeatedly until a clear filtrate is obtained. The clear filtrate is then seen with the TLC plate 15%, TBA 0.37% solution in HCl 0.25N, aquadest and drug inhalation.

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The ethanol extract of mustard leaf was identified by GC-MS using the appropriate working parameters. The spectrum obtained is compared with the GC-MS database spectrum. Wistar rats were grouped randomly, group P0 was only given distilled water as normal and group P1 was given glibenclamide as positive control. Group P2, P3, P4 were given green mustard extract at a dose of 0.5 mg / kg BW, respectively; 2.0 mg / kg body weight; 5.0 mg / kg body weight. Giving extracts given for 30 days. At the end of the stage, blood glucose level and 8-OHdG levels are tested. Measurement of 8-OHdG levels using blood taken from the Wistar rat’s eye. Measurement of 8-OHdG levels using the UV-Vis Spectrophotometry instrument with 8-OHdG DNA Damage Elisa Kit Cell Biolabs with the following steps: 1) Prepare all reagents before use; 2) Then add 50 µL of the sample to be examined and the standard 8-OHdG to 8-OHdG Conjugated Coated Plate. Incubate at room temperature for 10 minutes. 50 µL anti-8-OHdG was added, incubation for 1 hour. Guided by the lemar assay layout. Insert with a standard 100 L column diluent pipette (Assay buffer tissue culture media), add 250 µl of TCA reagent and 250 µl of TBA reagent then firmly vortex. The solution was incubated for 1 hour at 60°C and then centrifuged at 10,000 rpm for 2-3 minutes. Absorbance is read at maximum wavelength.

The sample measurements are carried out in the same way as in making standard solutions. The research data are statistically analyzed using the ANOVA method with the SPSS (Statistical Product and Services Solution) program using SPSS software for windows with a confidence level of 95%. Data analysis of the results of the study was carried out with the following stages:

The normality test is done by the Shapiro Wilk method (sample <30) which is used to test the normality of the data generated in the study. The data obtained were normally distributed if p > 0.05. Test the homogeneity of variance between groups with Leven’s, Comparative test to determine the effect between giving ethanol extract of agarwood leaves on blood sugar levels in hyperglycemic wistar rats using One Way ANOVA). After the normality test, homogeneity test and comparative test, to find out which groups have the same or different influence from one another do Post Hoc Study with LSD test [9].
3. Results and Discussion

Green mustard extraction: Green mustard extracted by maceration using 96% ethanol solvent. The ethanol solvent used is polar, so that the active substance in polar mustard greens can be attracted by the solvent. Ethanol was chosen as a solvent because it is safe and non-toxic. Ethanol 96% is used because it is volatile compared to water. The result of the amendment from the green mustard powder was obtained 6.9% (w/w), from the mass of simplicia to 1000 grams and resulting in a thick ethanol extract of 68.9 grams. Yield was calculated to determine the ratio of the amount of extract produced by simplicia. Comparative tests to determine the effect between giving ethanol extract of agarwood leaves on blood sugar levels in hyperglycemic wistar rats using an unvariant test (One Way ANOVA). After the normality test, homogeneity test and comparative test, to find out which groups have the same or different influence from one another do Post Hoc Study with LSD test [9].

Figure 1 Fraction Chromatogram.

Table 1. Compound Active from GC-MS (Gass Chromatography Massfactor)

| Peak | Tr  | % area | M+  | Compound                                      |
|------|-----|--------|-----|-----------------------------------------------|
| 1    | 4.41| 23.90  | 100 | Vinyl propionate                              |
| 2    | 4.52| 2.85   | 73  | Butyl formate                                 |
| 3    | 13.60| 2.29  | 150 | 2-Methoxy- 4-vinylphenol                      |
| 4    | 17.90| 1.71  | 194 | 13-xaxadispiro[5.0.5.1] tridecan-1-one         |
| 5    | 19.68| 19.68 | 178 | Methyl iso-eugenol 1                          |
| 6    | 20.14| 20.14 | 166 | Phenol. 3-isopropoxy-5-methyl-                |

Glibenclamide was chosen as a comparison of ethyl acetate fraction of green mustard ethanol extract because it can stimulate insulin secretion in the pancreas gland. The dose of glibenclamide used is 0.1 mg / 200 gbb. The dose is used based on the calculation of HED (Human Equivalent Dose) which is 5 mg which is then converted to rat doses. In this study it was found that administration of ethyl acetate fraction of mustard extract with various doses can reduce blood glucose levels in hyperglycemic wistar rats caused by alloxan induction. Aloxan will reduce insulin production so that it can cause an increase in blood glucose levels or hyperglycemia. Increased blood glucose levels are also caused due to the degeneration of β cells in the pancreas gland causing impaired insulin production resulting in insulin deficiency. Decreased insulin hormone causes all glucose consumed by the body can not be
processed perfectly, as a result, blood glucose levels increase in the body. Based on the test results shown in Figure 2.

![Graph showing decreases in 8-OHdG levels](image)

**Figure 2.** Decreases 8-OHdG (8-Hydroxy-2-dioxiguanosine).

Note: P0: Normal  
P1: Control (Glibenklamid)  
P2: Group consumption the dose of ethanol extract mustard greens ethyl acetate fraction 0.5 mg/kgbw  
P3: Group consumption the dose of ethanol extract mustard green ethyl acetate 2.0 mg/kgbw  
P4: Group consumption the dose of ethanol extract mustard green ethyl acetate 5.0 mg/kgbw

In the research of 8-OHdG reduction in Wistar hyperglycemia rats that were given ethanol extract of mustard greens ethyl acetate fraction. Streptozotocin will reduce insulin production so that it can cause an increase in blood glucose levels or hyperglycemia. Increased blood glucose levels cause an increase in free radicals and decreased antioxidant activity. Increased free radicals are characterized by increasing levels of 8-OHdG [3].

Based on the test results shown in Figure 2 shows that the higher the dose given is at a dose of 5.0 mg/kgbw the higher the decrease in df 8-OHdG levels in rats on the 3rd day after streptozotocin induction has an average of 8-OHdG 3.93 mg/dL and on day 14 decreased 2.99 mg/dL to 2.15 mg/dL. Whereas at dose kg and 15 mg/kg decreased by 3.43 mg/dL and 4.99 mg/dL.

This shows that the ethyl acetate fraction of mustard extract at a dose of 20 mg/kg the ability is almost the same as positive control (glibenclamide) which decreased the average blood glucose level of 6.97 mg/L. Whereas in normal mice there was an increase of 0.13 mg/dL.

Based on Figure 2 the results can be said that the ethyl acetate fraction of mustard extract can significantly reduce the 8-OHdG levels of hyperglycemic mice. This happens because it is influenced by the quantity of phenol compounds in the extract. Phenol compounds can work as antioxidants that can reduce oxidative stress so that there is a decrease in levels of 8-OHdG. Data Analysis 8-OHdG Decreased Levels in Wistar Rats The measurement results of decreased blood glucose levels were analyzed using statistical tests. The initial statistical test carried out is the normality test using the Kolmogorov-Smirnov and Shapiro-Wilks test, from the normality table in Appendix 11 it is known that the results of a decrease in blood glucose are normally distributed (p ≥ 0.05). The normality test aims to test whether the data obtained from each group is normally distributed (symmetric).

The next analysis is a homo Geneity test with the aim of testing whether the data obtained from each group has homogeneous variants. significant difference between negative control to positive control, 0.5 mg/kgBW dose treatment, 2.0 mg/kgBW and 5.0mg/kgBB treatment showed that treatment with ethanol extract of mustard greens ethyl acetate fraction could reduce levels of 8-OHdG in hyperglycemic Wistar rats. While the treatment dose of 5.0 mg/kgBw had no significant difference, which can be interpreted as both having the most significant effect of reducing 8-OHdG levels in the
administration of ethanol extract of mustard leaves 5mg/kgbw, due to polyphenol compounds such as flavonoids, polyenes, and compounds that contain lots of -OH group are multifunctional which can react with free radicals as: (a) reducing agent; (b) free radical scavengers; (c) chelating metal [10]. Ethanol extract of mustard leaf (Brassica rapa L) dose 5.0 mg/kgBw has the highest ability to decrease 8-OHdG activity. This is presumably due to the content of phenol compounds, flavonoids, and terpenoids which cause the highest synergistic work, thus causing the highest decrease in 8-OHdG.

4. Conclusions
Based on the research results obtained can be concluded as follows: 1). The administration of green mustard (Brassica rapa L.) ethanol extract at a dose of 5.0 mg / KgBw can reduce levels of 8-OHdG in Wistar rats that are hyperglycemic significantly (p <0.05) to negative controls; 2). Phenol compound is a compound which is proven to be contained in a large amount of ethanol extract of mustard greens (Brassica rapa L.) ethyl acetate fraction as a powerful antioxidant to cell damage (8-OHdG) in Wistar hyperglycemia rats. Further research needs to be done. Regarding the isolation and identification of active compounds in green mustard ethanol extract (Brassica rapa L.) leaf which can be anti-inflammatory along with acute toxicity in the administration of ethanol extract of mustard leaf (Brassica rapa L) in Wistar rats.

References
[1]. PERKENI, 2012, Konsensus Pengelolaan Diabetes pada Diabetes Melitus tipe 2. PB Perkeni. Jakarta
[2]. Tjokroprawiro, A., 1993, Radikal Bebas, Aspek Klinik dan Kemungkinan Aplikasi Terapi, in : Simposium Oksidan dan Antioksidan, Tjokroprawiro Edt, Persatuan Ahli Penyakit Dalam Cabang Surabaya, hal 11-36
[3]. Fitriana Mahmudah, K., 2011, Uji Aktivitas Antidiabetes dengan Metode Penghambatan Enzim α-Glukosidase dan Skrinning FitoKimia Pada Beberapa Tanaman Indonesia, FMIPA Universitas Indonesia, Depok.
[4]. Masharani, U., Karam, J.H., and German, M.S., 2004, Pancreatic Hormones & Diabetes Mellitus, Greenspan FS, Gardner DG. (Ed), Basic & Clinical Endocrinology, 7th Ed, McGraw-Hill, New York, 658-746
[5]. Tirosh, A., Rudich, A., and Bashan, N., 2000, Regulation of Insulin Transporters Implication for Insulin Resistance States, J. Pediatr Endocrinol metab, 13 : 115-19
[6]. Azizah, T.S., dan Munawaroh, R., 2009, Interaksi Quercetin Dengan Tolbutamid: Kajian Terhadap Perubahan Kadar Glukosa Darah Pada Tikus Jantan Yang Dinduksi Aloksan, Jurnal Penelitian Sains & Teknologi, Vol 10.
[7]. Szkudelski, T., 2001, The Mechanism of Alloxan and Streptozotocin Action in B Cells of the Rat Pancreas, Physiol. Res, 50: 536-546
[8]. Suherman S.K., 2007, Insulin dan Antidiabetik Oral. Dalam : Gunawan, S.G. Farmakologi dan Terapi. Edisi 5, Jakarta: Balai Penerbit FKUI. 485; 489-93.
[9]. Pramesti, G., 2007, Aplikasi SPSS 15.0 dalam Model Linier Statistika, Penerbit PT Elex Media Komputindo, Jakarta
[10]. Akhlaghi, M., dan Brian, B., 2009, Mechanisms of Flavonoid Protection Against Myocardial Ischemia-reperfusion injury”, Journal of Molecular and Cellular Cardiology, 46 : 309-17