

GREEN TEA EXTRACT EFFECT ON BLOOD GLUCOSE LEVEL AND LIVER HISTOPATHOLOGY IN DIABETIC MICE

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INTRODUCTION

Diabetes mellitus is a disorder of hyperglycemia and glucose intolerance due to insulin deficiency, impaired of insulin receptor or both (Unwin *et al.*, 2009). There are generally two types of diabetes are type 1 diabetes (pancreatic beta cell damage caused absolute insulin deficiency) and type 2 (a combination of a lack of insulin production and secretion and sensitivity to insulin receptor) (Dipiro *et al.*, 2008).

Diabetes mellitus disease is increasing rapidly in worldwide. The incidences in 2010 were about 285 million people and It has been estimated that by the year 2025, the global incidence of diabetes would increase to 350 million (International diabetes federation, 2006).

In diabetes, activation of hepatic gluconeogenesis enzymes can increase glucose production and thus contribute to increase blood glucose which could deteriorate diabetes (Sundaram *et al.*, 2013). The state of diabetes characterized by decreased insulin sensitivity is the major cause of NAFLD (Non - Alcoholic Fatty Liver Disease), because in diabetes state occurs disorders of glucose metabolism and fat so that could result in fibrosis, infiltration, necroinflammation, to acute liver disease (Marchesini *et al.*, 2001).

Treatment of diabetes mellitus is chronic and long life, causing undesirable side effects (Unwin *et al.*, 2009). Metformin is an oral hypoglycemic agent, which belongs to the class known as the biguanides. Metformin is now widely used as one of the mainstays in the management of type 2 diabetes. Metformin reduces fasting plasma glucose concentration by reducing rate of hepatic glucose production via gluconeogenesis and glycogenolysis. Metformin improves glycemic control as monotherapy and in combination with other oral antidiabetic agents, such as sulfonylureas and thiazolidinediones (Frendell *et al.* 2003).

Several plant extracts are known to have antidiabetic properties and a large number of compounds from plant extracts have been reported to have beneficial effects for treatment of diabetes mellitus (Anhauser, 2003). Tea (*Camellia sinensis* L.) is one of plant that can decrease blood glucose. Green tea is produced

by enzymatic inactivation of the leaves of *Camellia sinensis* followed by rolling or comminution and drying. In the manufacturer of green tea, the enzymatic inactivation achieved by steam or pan firing treatment to preserve natural polyphenols with respect to the health promoting properties. Green tea derived products are mainly extracts of green tea in liquid or powder form varying in the proportion of polyphenols (45-90%) and caffeine content (0.4-10%). The polyphenolic fraction of green tea, has been reported to have multiple pharmacological actions (Sano *et al.*, 1995).

Green tea is an excellent source of polyphenol antioxidants, known as green tea catechins. The important catechins of green tea are epicatechin (EC), epicatechin-3-gallate (ECG), epigallocatechin (EGC) and epigallocatechin-3-gallate (EGCG). The polyphenolic fractions of green tea have been reported to have multiple pharmacological actions. They exhibit potent antioxidant activity in vitro and in vivo. Epidemiologic observation and laboratory studies have indicated that polyphenolic compounds present in the tea may reduce the risk of a variety of illnesses, including cancer and coronary heart disease (McKay and Blumberg 2002).

Some studies suggest that green tea extract lowered cholesterol levels and blood glucose on mice and rat (Yang *et al.*, 2001). Green tea extract at dose of 300mg /kg /day can lower blood glucose in diabetic rats and was also able to reduce the lipids in heart defects (Babu *et al.*, 2006). Blood glucose lowering activity of green tea was greater and total polyphenol content was higher when compared with black tea and oolong tea (Holidah *et al.*, 2015).

METHODS

Animal

Adult male Balb/C mice (20-30 g and 2-3 month age) were kept in mice cages at room temperature ($27 \pm 2^{\circ}\text{C}$) and humidity ($55 \pm 5\%$) and a 12 hours cycle of light and dark. The mice were acclimatized for two weeks prior to commencement of the experiment and supplied with standard pellet food with tap water *ad libitum*.

Chemical

Green tea (PT Perkebunan Nusantara XII), alloxan monohidrat (TCI), metformin hydrochloride (Sigma-Aldrich), CMC-Na 1%, NaCl 0,9%, aquabidestilata, formalin 10%, alcohol, xylol, parafin, Hematoxyllin Eosin (HE).

Preparation of Green Tea Extract

100 g dried green tea was soaked with 1000 ml hot water for 15 minutes. Filtrate were filtered by Vacuum and Whatman filter paper. The extract were dried on freeze drier below -80°C.

Experimental Procedure

Induction of diabetes in mice

Diabetic mice induced by alloxan monohidrat 225mg/KgBW. After 3 days the mice showing diabetes having blood glucose values more than 200 mg/dL was considered as diabetic animals consider it as zero days. The mice were divided into 5 treatment groups. Alloxan diabetic group, extract treated groups (3) and metformin treated group.

Blood glucose measurement

Dosing with the extracts (300, 600 and 1.200 mg/KgBW) and metformin (110 mg/kbBW) were started on the first day and continued for 14 days. Blood was collected on 15th day of treatment. Blood was collected retro-orbitally from the inner cantus of the eye (under light ether anesthesia) using capillary tubes. Blood glucose level was determined by using enzymed GOD-POD glucose kit using colorimeter. Data of blood glucose levels was analyzed by one way ANOVA followed by LSD post hoc multiple comparsion test.

Histopathological study

The liver tissues were collected and fixed in phosphate buffered formalin at room temperature overnight then dehydrated in ethanol (concentration 80%, 95% and 100%). The liver cleaned with xylene, cut with a 4 mm thick, deparaffinated, then washed again with water and stained with Hematoxylin eosin (HE) (Tang *et al.* , 2013) and then examined under a microscope with a magnification of 400 times for morphological analysis.

RESULTS AND DISCUSSION

Result

After alloxan induced, blood glucose levels of mice were measured, the diabetic glucose levels occur on day 5 for all groups. Blood glucose levels were increased on day 2nd and the peak occurred on day 5th after alloxan induction (Sujono, 2010).

Diabetogenic agent Alloxan is destructive pancreatic β-cells and cause pancreatic disorders in insulin production resulting in the increase in mice’s blood glucose levels. The formation of reactive oxygen species are the main mechanism of action of alloxan in β-cell damage. Alloxan ruined the DNA of pancreatic β-cells so that the granules storage reduced insulin which causes a deficiency of insulin in the end there was an increase in blood glucose (Nugroho, 2006).

Green tea extract decreased blood glucose level of diabetic mice after 14 day of treatment. Green tea extract dose of 300 mg/kg BW lowered blood glucose level less effective than green tea extract dose 600 and 1.200 mg/kgBW and metformin group. Green tea extract dose 600 mg/kgBW had biggest blood glukosa level reduction than other group but not significantly different with green tea extract dose 1.200 mg/kgBW group (see Table 1 and Figure 1).

Table 1. decrease blood glucose level after treatment

group	decrease of blood glucose level (%)
k+	35.27±4.97 ^a
d300	19.63±2.55 ^b
d600	64.34±1.27 ^c
d1200	63.43±7.45 ^c

Values are given as mean ± S.D. for six animals in each group (n = 6). Values are considered significantly different at p < 0.05 with post hoc LSD test.

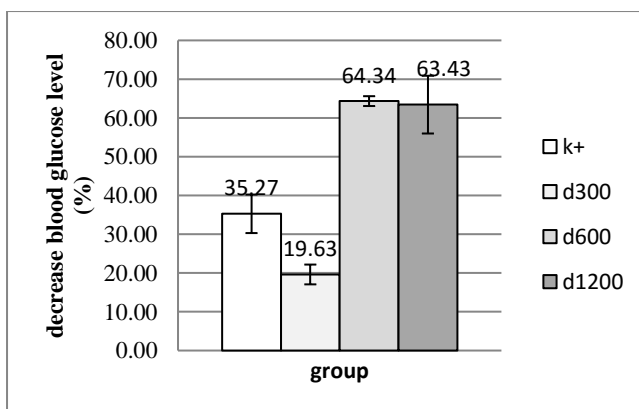


Figure 1. Decrease blood glucose level after treatment

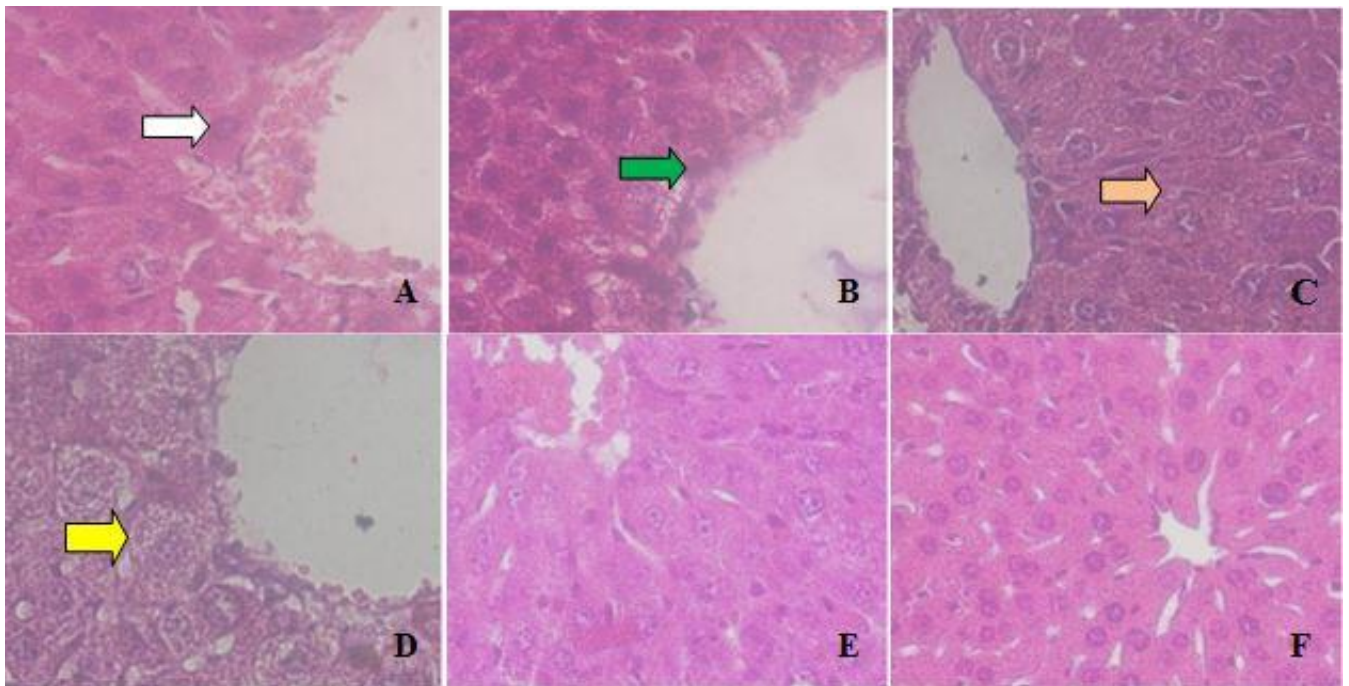


Figure 2. Liver Histopathology of Mice, when (A) Normal, (B) negative control, (C) positive control, (D) Green tea extract dose 300 mg/kgBB, (E) 600 mg/kgBB, (F) 1.200 mg/kgBB. Normal hepatocyte (), Parenchymatosa degeneration (), Hydropic degeneration (), and Necrosis (). Magnification 400X.

Group of diabetic have irregular hepatocytes structure because many cells degenerate parenchymatosa, hydropic degeneration, and necrosis. The liver of negative control most damage because diabetes can induce proinflammatory cytokines with the increased levels of tumor necrosis factor alpha (TNF- α), interleukin-1 (IL-1) and interleukin-6 (IL-6) which causes inflammation that can increase liver damage (Chen *et al.*, 2015). Hepatocytes structures of positive control group arranged radially compared with the diabetic group. The hepatocytes structure of group of green tea extract dose of 600 and 1,200 mg/kgBW more regular than the dose group of 300 mg/kgBW (see Figure 2).

DISCUSSION

The results of this study showed that green tea extract has the ability to decrease blood glucose levels and improve diabetic mice liver damage. Green tea extract and metformin significantly decrease the activity of enzymes gluconeogenesis in the liver, increases the sensitivity of the absorption of glucose into cells and improve cell- β damaged, and green tea extract also increases glucose uptake in adipose tissue of mice (Babu *et al.*, 2006; Sundaram *et al.*, 2013; Tang *et al.*, 2013). This is due to the polyphenolic compounds contained in green tea leaves. Polyphenols in green tea can increase the absorption of glucose in the intestines and increases the activity of insulin, thereby reducing blood glucose in mice with type 1 and 2 DM (Babu *et al.*, 2006)

Flavonoids also act as antioxidants by keeping the β -cells from the toxic effects caused by alloxan and is also able to repair damaged liver cells (Tang *et al.*, 2013). Tea is known to contain flavonoids called catechins, believed to have the effect reducing blood glucose levels and improve hepatic DM mice. Catechins in green tea extract can increase the activity of the insulin receptor in adipose tissue and serves as a catcher of free radicals. In the diabetic state of free radicals to spread via the blood to adipose tissue such as liver damage and one trigger for insulin resistance in tissues. As a powerful antioxidant catechins can improve TNF- α and can reduce free radicals in the blood and increases resistance to the insulin receptor directly inhibit the glucose transporter (GLUT-1) and increasing the activity of GLUT-4 in the cell membrane (Yan *et al.*, 2012).

CONCLUSION

The results showed that green tea extract dose of 600 mg/kg BW lowered blood glucose level more effective than 300 and 1.200 mg/kg BW in alloxan induced diabetic mice. Green tea extract restore the morphological change of liver tissue in the diabetic state and turning to the normal state after repeated treatment.

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