

## Oolong Tea Infusion Lowers Blood Glucose in Alloxan-Induced Diabetic Mice

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### Abstract

Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia and associated systemic and oral complications. Plant-based therapies are increasingly studied as complementary options for improving glycemic control, and oolong tea (*Camellia sinensis*), which contains antioxidant polyphenols, has been proposed to possess antidiabetic activity. This study evaluated the glucose-lowering effect of oolong tea infusions at concentrations of 10%, 20%, and 40% in alloxan-induced diabetic mice. Male mice were divided into five groups, including negative and positive controls and three treatment groups receiving the respective infusions. Diabetes was induced with alloxan (16.8 mg/kg), and baseline blood glucose was measured on day three. On day five, mice received oral administration of oolong tea infusion (0.25 mL/25 g), followed by blood glucose measurement one hour later. Alloxan successfully induced hyperglycemia, and the 10% and 20% oolong tea infusions significantly reduced blood glucose levels to values comparable to healthy controls. The 40% infusion produced a smaller reduction, suggesting diminished bioactivity at higher concentration, likely due to polyphenol degradation and higher caffeine content. These findings indicate that oolong tea infusion has meaningful antidiabetic potential in this experimental model, with moderate-strength preparations showing the strongest effect. Oolong tea may offer a complementary approach for supporting glycemic control, although further studies are needed to clarify optimal dosing and underlying mechanisms.

**Keywords:** Diabetes; Hyperglycemia; Oolong Tea; Glycemic control

### 1. Introduction

The oral cavity is highly susceptible to infection because it harbors a diverse population of microorganisms. Conditions observed in the oral cavity can also reflect a person's systemic health. Diabetes mellitus, a systemic disorder affecting carbohydrate metabolism, results in persistently elevated blood glucose levels and increases the risk of major lesions involving oral cavity tissues, supporting bone, and periodontal connective structures [1].

In Indonesia, diabetes has shown a notably high and increasing prevalence. From 1964 to 2003, the number of patients with diabetes mellitus receiving treatment at Dr. Soetomo Hospital, Endocrinology Clinic, increased nearly 300-fold—from 133 to 39,875 cases—with an average rise of approximately 1,022 new patients per year. According to epidemiological estimates from the IDF in 2003, Indonesia ranked sixth globally in the number of individuals with diabetes [2]. Preventing diabetes and reducing its complications therefore remain major public health priorities. Improving glycemic control, lowering cholesterol, and managing blood pressure can reduce diabetes-related cardiovascular mortality by up to 30%. Oral infections such as periodontal disease further complicate diabetes, as

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inflammation worsens glycemic control, and poor glycemic control exacerbates periodontal breakdown. This bidirectional relationship underscores the importance of maintaining oral health in diabetic patients. In fact, the American health system has set a target for 71% of individuals with diabetes to undergo annual dental examinations [3].

Most antidiabetic medications are costly and require long-term use, which may lead to adverse side effects. For this reason, medicinal plants are gaining interest as complementary options for diabetes management. Tea leaves (*Camellia sinensis*) represent one such potential natural therapy. Oolong tea, a partially fermented type of tea, has been suggested as an alternative approach for helping to lower blood glucose levels. Epidemiological findings indicate that populations with habitual oolong tea consumption tend to have reduced blood glucose levels, suggesting possible antidiabetic benefits. Further experimental evidence, however, is needed to support this observation [4].

Tea is generally classified into three categories—green tea (non-fermented), oolong tea (semi-fermented), and black tea (fully fermented)—with the degree of fermentation altering the polyphenol content of the leaves. Green tea contains the highest polyphenol concentrations and has been studied extensively for antidiabetic effects [5]. Based on this, the present study aims to determine whether oolong tea, despite its partial fermentation and comparatively lower polyphenol levels, still demonstrates meaningful antidiabetic activity. Oolong tea retains bioactive catechins and flavanols that function as potent antioxidants capable of reducing the free radicals generated during alloxan-induced  $\beta$ -cell damage, making it a promising candidate for investigation.

## 2. Material and methods

### 2.1. Preparation of Oolong Tea Infusions

Dried oolong tea leaves (*Camellia sinensis*) were used to prepare infusions at concentrations of 10%, 20%, and 40%. A 10% infusion was prepared by weighing 10 g of dried leaves and adding 100 mL of distilled water. The mixture was heated until it reached 90°C, maintained for 15 minutes, then removed from heat and allowed to cool. The infusion was filtered, and the volume was adjusted back to 100 mL with distilled water.

To obtain the 20% infusion, the 10% extract was heated at 40°C until the volume was reduced to approximately half of the initial volume. The 40% infusion was prepared by further concentrating the 20% infusion using the same procedure until the volume was again reduced by half. All infusions were freshly prepared on the day of administration to maintain stability.

### 2.2. Animals and Experimental Design

Male mice were acclimatized to the laboratory environment for seven days prior to experimentation. After acclimatization, animals were randomly assigned into five groups (n = 5 mice/group):

- Negative control: Healthy mice receiving distilled water
- Positive control: Alloxan-induced diabetic mice receiving distilled water
- Treatment I: Alloxan-induced mice receiving 10% oolong tea infusion
- Treatment II: Alloxan-induced mice receiving 20% oolong tea infusion
- Treatment III: Alloxan-induced mice receiving 40% oolong tea infusion

Diabetes was induced in all groups except the negative control by a single intraperitoneal injection of alloxan at 16.8 mg/kg body weight, following the dose conversion described by Chacko (2007) [6]. Blood glucose levels were measured from the tail vein three days after alloxan injection to confirm hyperglycemia.

### 2.3. Treatments

On day five, mice in the treatment groups received the respective oolong tea infusions orally at a dose of 0.25 mL/25 g body weight. The negative and positive control groups received distilled water in an equivalent volume. Blood glucose levels were re-measured one hour after administration.

### 2.4. Blinding Procedures

To minimize bias, two levels of blinding were employed:

- Treatment blinding: Infusions and control solutions were prepared and coded by an investigator who was not involved in outcome measurement. The researcher administering treatments and recording data was unaware of group allocation.
- Outcome blinding: Blood glucose measurements were performed by an independent investigator using coded animal IDs. The code key was revealed only after all data collection was complete.

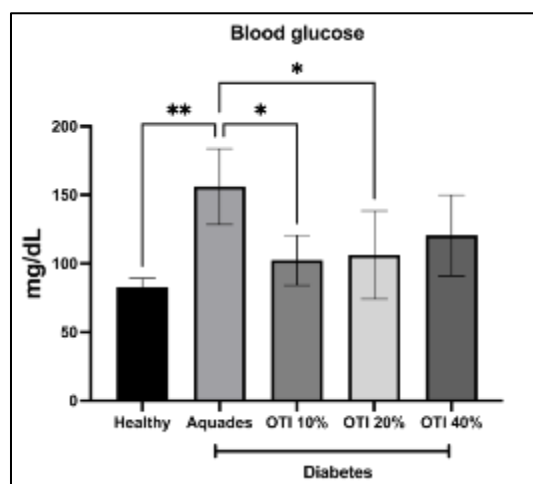
## 2.5. Statistical Analysis

Data were analyzed using one-way ANOVA to compare mean blood glucose levels among groups. When a statistically significant difference was detected, the analysis was followed by an LSD post hoc test. Statistical significance was set at  $p < 0.05$ .

## 3. Results and discussion

This study demonstrates that oolong tea infusion exerts antidiabetic activity in alloxan-induced mice, with the 10% and 20% concentrations showing the strongest glucose-lowering effect. Alloxan selectively destroys pancreatic  $\beta$ -cells through free radical generation, leading to insulin deficiency and hyperglycemia. The improvement observed after administration of oolong tea supports the hypothesis that its bioactive compounds can counteract oxidative stress and enhance glucose regulation [7].

Induction of diabetes with alloxan successfully elevated blood glucose levels in mice compared with the healthy control group. The negative control (healthy mice given aquadest) showed a mean blood glucose concentration of  $82.86 \pm 6.41$  mg/dL. In contrast, the positive control (alloxan-induced diabetic mice given aquadest) demonstrated a marked increase to  $156.14 \pm 27.73$  mg/dL, confirming effective hyperglycemia ( $p < 0.05$ ).



**Figure 1** Mean blood glucose levels in mice after three days of treatment with oolong tea infusion at concentrations of 10%, 20%, and 40%. Alloxan-induced diabetic mice showed a marked increase in glucose level compared with healthy controls. Administration of 10% and 20% oolong tea infusions reduced blood glucose toward near-normal levels, while the 40% infusion produced a weaker glucose-lowering effect. Bars represent mean  $\pm$  SD. Different superscript letters indicate statistically significant differences between groups (ANOVA followed by LSD post hoc test,  $p < 0.05$ ).

OTI- Oolong tea Infusion

Administration of oolong tea infusion for three days produced a glucose-lowering effect across all tested concentrations. Mice treated with 10% and 20% oolong tea infusions exhibited mean glucose levels of  $102.43 \pm 18.21$  mg/dL and  $106.29 \pm 32.15$  mg/dL, respectively. These values were significantly lower than the diabetic control and were statistically comparable to the healthy control group, indicating partial normalization of glycemia.

In contrast, the 40% oolong tea infusion group showed a mean glucose concentration of  $120.57 \pm 29.53$  mg/dL, which remained significantly higher than the 10% and 20% treatment groups and closer to the diabetic control level ( $p < 0.05$ ). This suggests a diminished antihyperglycemic effect at the highest concentration.

Taken together, these findings show that short-term administration of oolong tea infusion at 10% and 20% concentrations effectively reduced alloxan-induced hyperglycemia, while the 40% concentration did not provide additional benefit and appeared less effective.

Alloxan was chosen as the hyperglycemia-inducing agent because of its selective toxicity toward pancreatic  $\beta$ -cells. Its structural similarity to glucose allows rapid uptake into  $\beta$ -cells, where it generates free radicals that lead to  $\beta$ -cell destruction and impaired insulin secretion [8]. This mechanism replicates the metabolic disturbance characteristic of diabetes mellitus, in which insufficient insulin causes elevated blood glucose levels and contributes to long-term systemic complications [9].

Oolong tea reduced blood glucose levels across all treatment concentrations, indicating antidiabetic potential. The improvement observed with the 10% and 20% infusions is consistent with the known biological activity of tea polyphenols. Oolong tea contains catechins such as EC, EGC, GCG, and EGCG, which serve as potent antioxidants capable of scavenging free radicals produced during alloxan exposure [10]. These polyphenols also enhance endogenous antioxidant enzymes such as superoxide dismutase, helping preserve  $\beta$ -cell function [11,12]. Beyond antioxidant activity, tea polyphenols exert additional antidiabetic effects, including inhibition of  $\alpha$ -amylase and  $\alpha$ -glucosidase, modulation of endothelial and immune pathways, and reduction of oxidative stress [11].

Polyphenols—especially EGCG—also influence glucose metabolism by improving insulin receptor phosphorylation and increasing glucokinase expression, which promotes glucose uptake and energy production. This process increases intracellular calcium levels and stimulates insulin release [12]. Other compounds in oolong tea, including flavonoids and caffeine, can further support glucose regulation and  $\beta$ -cell recovery [13,14].

The reduced effectiveness of the 40% infusion may be explained by changes in chemical stability at higher concentrations. Excessive heating during preparation can degrade polyphenols, reducing their antioxidant capacity [15]. Higher caffeine content may also counteract beneficial effects by decreasing insulin sensitivity through increased epinephrine release [16]. Additionally, the more alkaline pH of the 40% infusion could limit passive diffusion of bioactive compounds across cell membranes [7], lowering their biological activity despite a higher concentration of tea extract.

Recent evidence supports the mechanisms proposed in this study. EGCG protects  $\beta$ -cells by suppressing oxidative stress and abnormal autophagy [17], and tea phenols stimulate postprandial insulin release [18, 19]. Oolong tea also enhances insulin sensitivity through activation of the PI3K/Akt pathway and GLUT4 translocation [20], improves hepatic glycogen synthesis, and reduces insulin resistance via the INSR-IRS-PI3K-AKT signaling network [21,22]. Its ability to inhibit carbohydrate-digesting enzymes further contributes to reduced postprandial glucose spikes [20].

Overall, these findings indicate that oolong tea lowers blood glucose through multiple synergistic mechanisms, including  $\beta$ -cell protection, enhancement of insulin signaling, activation of metabolic pathways, and inhibition of carbohydrate digestion.

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#### 4. Conclusion

This study shows that oolong tea infusion has meaningful antidiabetic potential in alloxan-induced mice, with the 10% and 20% concentrations producing the most consistent improvement in blood glucose regulation. These effects appear to stem from preserved polyphenol activity and favorable impacts on  $\beta$ -cell protection, insulin signaling, and oxidative stress. In contrast, excessive concentration of the infusion may reduce its bioactivity, as reflected by the weaker response at 40%. Overall, the findings indicate that moderate-strength oolong tea infusion is more effective than highly concentrated preparations in attenuating hyperglycemia. Further studies using longer treatment duration, standardized polyphenol quantification, and molecular assays are needed to confirm the mechanisms and to determine whether these benefits can be translated into therapeutic or dietary applications.

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#### Compliance with ethical standards

The protocol of this study was received ethical approval from the Ethics Committee, Animal Care and Use Committee Faculty of Veterinary, Universitas Airlangga (Register Number: 1.KEH.10.02.2025). All efforts were made to minimize animal discomfort, including the use of appropriate anesthesia during tissue collection.

### Disclosure of conflict of interest

No conflict of interest to be disclosed.

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