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Antihyperglycemic Activity of Camellia Sinensis on Alloxan Induced Diabetes Mellitus in Male Wistar Albino Rats

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Author`s Contribution

[1,3]Substantial contributions to the conception or design of the work; or the acquisition, [5,6]Active participation in active methodology,[2]analysis, or interpretation of data for the work, [4,6]Drafting the work or revising it critically for important intellectual content

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ABSTRACT

Objective: To evaluate the antihyperglycemic effect of Camellia sinensis in alloxan-induced diabetes mellitus using a male Wistar albino rat model.

Methodology: This experimental study was conducted at the Department of Biochemistry, Liaquat University of Medical and Health Sciences (LUMHS), Jamshoro, from September 2022 to March 2023. Sixty adults male Wistar albino rats were randomly allocated into three groups (n=20 each): Group A (negative control) received normal saline, Group B (positive control) received alloxan (120 mg/kg, intraperitoneally), and Group C received alloxan (120 mg/kg, intraperitoneally) followed by Camellia sinensis (100 mg/kg/day) for 30 days. Diabetes was confirmed by blood glucose levels ≥250 mg/dL after 72 hours of alloxan administration. At the end of the experiment, blood samples were collected for estimation of random blood glucose (RBG) and fasting blood glucose (FBG). Data were analyzed using one-way ANOVA followed by Fisher's LSD post hoc test. A p-value ≤0.05 was considered statistically significant.

Results: Mean RBG was significantly lower in the Camellia sinensis-treated group (323.15±9.91 mg/dL) than in untreated diabetic rats (499.05±34.29 mg/dL) (F=1570.9, p=0.0001). Similarly, mean FBG was significantly reduced in Group C (116.30±9.89 mg/dL) compared with Group B (146.30±37.14 mg/dL) (F=44.7, p=0.001), although values remained higher than those of the normal control group.

Conclusion: Camellia sinensis significantly reduced fasting and random blood glucose levels in alloxan-induced diabetic rats, demonstrating promising antihyperglycemic activity. These findings support its potential as a complementary therapeutic agent for diabetes mellitus and warrant further clinical investigations in humans.

Keywords: Camellia sinensis, diabetes mellitus, alloxan, antihyperglycemic activity, Wistar rats, blood glucose.

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Introduction

Diabetes mellitus (DM) is a primary disorder of glucose metabolism characterized elevated blood glucose levels caused by defective insulin response. Chronic hyperglycemia is the hallmark of DM, may be associated with normal, low or elevated serum insulin levels.[1] This is caused by deficiency or inappropriately reduced insulin action. Pakistan shows high prevalence of DM. Estimating prevalence of DM is difficult in Pakistan due to lack of proper hospital records. Pakistan occupies 3rd position over the globe regarding the diabetes mellitus

prevalence.[2] DM is a multifactorial metabolic disorder. DM complications are attributed to the chronic hyperglycemic states causing glucose toxicity. Hyperglycemia mediated injury of target organs is the serious issue affecting the kidneys, retina, and nerve fibers, etc at the most.[3,4] Blood glucose varies moment to moment throughout the clock. Tissue demand, supply and hormone influence its homeostasis. Blood glucose in a fasting human being is <100 mg/dl. After meal intake (post prandial) it peaks to <200mg/dl. Green tea shows chemical substance beneficial for human health. Polyphenols, Theanine, tea polysaccharides, caffeine, and other substance show excellent biological activity. Anti – oxidant, anti – microbial, and anti – oxidant activity has been reported. Green tea lowers blood glucose, protects brain and minimizes oxidative agents. Efficacy of green tea has been reported against the atherosclerosis, diabetes mellitus, hyperlipidemia, systemic hypertenison and coronary artery disease, etc.[5,6] Blood sugar lowering effect of green tea is observed in diabetic tea consumers. Tea consumption prevents diabetes development in animal studies. It also delays the diabetes development. Blood glucose is regulated properly by the green tea. Regular tea consumption helps type 2 diabetes management. [7,8] Toxic side effect of green tea though relatively few but are narrated. Caution should be taken when consumed in elderly population, children and pregnant ladies.[9,10]

Available antihyperglycemic drugs have lots of side effects that's why there is a research gap and space for nutraceutical drugs to sufficiently decreases the complications of hyperglycemia with few to none side effects. This study is proposed to see the antihyperglycemic potential of Camellia Sinensis in high sucrose diet fed male wister albino rats.

Methodology

This experimental study was conducted over a period of six months following approval of the synopsis by the Ethics Review Committee. The research was carried out at the Department of Biochemistry, LUMHS Jamshoro from 29-9-2022 to 28-3-2023, while the animals were housed at Sindh Agricultural University, Tando Jam. All laboratory investigations were performed at the Diagnostic and Research Lab, LUMHS.

The study utilized sixty Albino Wistar rats as subjects, which were divided into three distinct groups for comparison purposes. The control rats were separated into negative and positive control groups, designated as Group A and Group B respectively, along with an experimental group labeled as Group C.

Group A served as the negative control and consisted of rats that received 0.9% normal saline as a placebo treatment. These animals were housed under standard conditions to establish normal baseline findings. Group B functioned as the positive control group and included diabetic rats that were induced with Alloxan at a dose of 120 mg/kg body weight administered intraperitoneally. These diabetic rats were left untreated throughout the study period to serve as positive controls for comparison.

The experimental Group C comprised diabetic rats that were also induced with Alloxan at 120 mg/kg body weight intraperitoneally, similar to Group B. However, these rats received additional treatment with Camellia sinensis at a dose of 100 mg/kg body weight. The Camellia sinensis therapy was administered continuously for a duration of thirty days to evaluate its therapeutic effects on the diabetic condition.

Alloxan monohydrate was purchased from the "Sigma Aldrich, St. Louis, MO, USA". It was stored at 4°C. Alloxan was prepared by dissolving it in normal saline at 27^0C. A night fasting rats were injected alloxan intraperitoneal (*i.p*.) route. Alloxan was buffered in 100 mM citrate buffer (pH 4.5). Dose was calculated 120 mg/kg.[11] Alloxan 120 mg/Kg bwt was injected *i.p.*[11] *in* supervision of a veterinary medicine specialist. Alloxan induces β-cells toxicity and produces frank diabetes mellitus within 48–72. A rat showing glucose value ≥250 mg/dl is defined as successful induction of DM.

In first phase, 20 rats were labelled as negative control group A (no diabetes induction, no therapy). 40 rats were selected by simple random sampling for Alloxan given at dose 120 mg/kg bwt intraperitoneal (*i.p*). Successful DM induction was defined as rats achieving glucose levels of >250 mg/dl at 72 hours interval. Random sampling used 20 diabetic rats allocated and labeled as positive control group B and left untreated. Remaining 20 diabetic rats were labelled experimental group C and treated with Camellia sinensis (100 mg/kg bwt/d) for 30 days.[49]

A sample of 60 Adult male albino Wistar rats was purchased according to inclusion and exclusion criteria.

Inclusion Criteria for negative control (Group A)

- Adult male albino Wistar rats,
- Body weight 150- 200gm body weight,

Inclusion Criteria for positive control (Group B) and experimental group (Group C),

- Adult male albino Wistar rats,
- Body weight 150- 200gm body weight,
- Rats achieving glucose level of >250 mg/dl at 72 hours of alloxan therapy,

Exclusion Criteria

- Female rats
- Old age, overweight male rats,
- Unsuccessful – induction of DM

Rats in groups A, B and C were handled for experiment as per protocol. At the end of experiment, rats were anesthetized by Ketamine (10 mg/Kg) and Xylazine (0.5 mg/Kg) as cited.[52] Blood samples were collected from all 3 groups (A, B & C) through cardiac puncture. Samples were taken in NaF (sodium flouride) – containing tubes and plain tubes. Samples were centrifuged and sera collected. Centrifugation was performed at 4°C, 5000 rpm, for 15 min. Supernatant was collected, put in tubes and stored at very low temperature (-80°C) for biochemical measurement of research variables.

Blood sera centrifuged were estimated for the biochemical assay using GLUC3 and ELECSYS INSULIN kits. Estimates were performed using Standard methods at the Diagnostic and Research Laboratory of Liaquat University.

1. Random Blood glucose levels
2. Fasting blood glucose levels

Approval of institute`s ERC (ethical review committee) of Liaquat University was taken for conducting the animal research study. Research proposal showing detailed protocol was presented and submitted for approval. Research proposal was submitted to the committee of Animal house of Sindh Agriculture University, Tando Jam. Protocol was approved unanimously. Animal handling was in accordance to the NIH Guideline & Local Institutional guideline.

Data was analyzed on SPSS version 22.0 (IBM, Incorp, USA). Data saved in Microsoft Excel sheet was copied and pasted on the SPSS generated sheet ready for analysis. Continuous variables examined for normal distribution. Variables were analyzed using one – way ANOVA (analysis of variance) and Fischer`s LSD post-Hoc testing. Level of statistical significance was at confidence interval 95% ($p \leq 0.05$).

Results

Results of random blood glucose (RBG) of 3 rat groups are shown in table I and figure 1. RBG in control group A is 123.1±8.78 mg/dl. Control group B shows RBG as 499.05±34.29 mg/dl. A significant decrease in RBG is shown in Camellia sinensis treated group C 323.15± 9.91 mg/dl. F value of 1570.9 and P-0.0001 shows significant differences among groups. RBG decrease in group C signifies the effectiveness of Camellia sinensis therapy (30 days).

Table I: Random Blood Glucose (mg/dl)

	Mean±SD	F- Value	P-value
Group A: Control rats– 0.9% N/saline	123.10± 8.78	1570.9	0.0001
Group B: Diabetic rats (Alloxan 120 mg/kg bwt)	499.05± 34.29		
Group C: Diabetic rats + C.sinensis (100 mg/kg bwt)	323.15± 9.91		

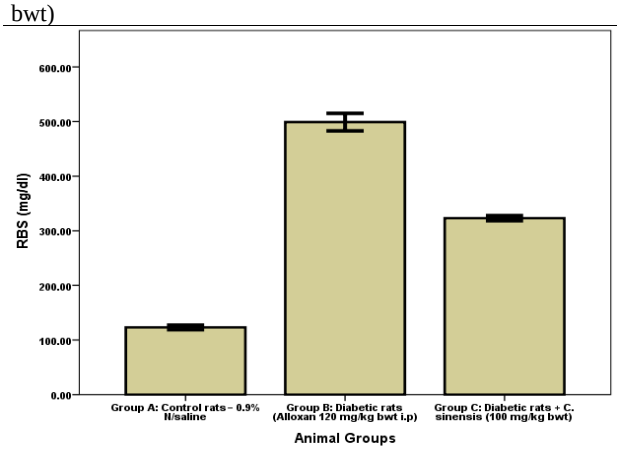

Figure 1. Bar graphs show random blood glucose levels in 3 rat groups. Compare with table I.

Fasting blood glucose (FBG) values are shown in table – 2 of 3 rat groups. FBG in control group A is 77.40±11.08 mg/dl. Control group B shows FBG as 146.30±37.14 mg/dl. Significant reduction of FBG is shown in Camellia sinensis treated group C 116.30± 9.89 mg/dl. F value of 44.7 and P=0.001. FBG reveals Camellia sinensis therapy (30 days) proved its biochemical action in reducing the glucose levels.

Table II: Fasting Blood Glucose (mg/dl)

	Mean±SD	F-Value	P-value
Group A: Control rats– 0.9% N/saline	77.40±11.08	44.7	0.001
Group B: Diabetic rats (Alloxan 120 mg/kg bwt)	146.30±37.14		
Group C: Diabetic rats + C.sinensis (100 mg/kg bwt)	116.30±9.89		

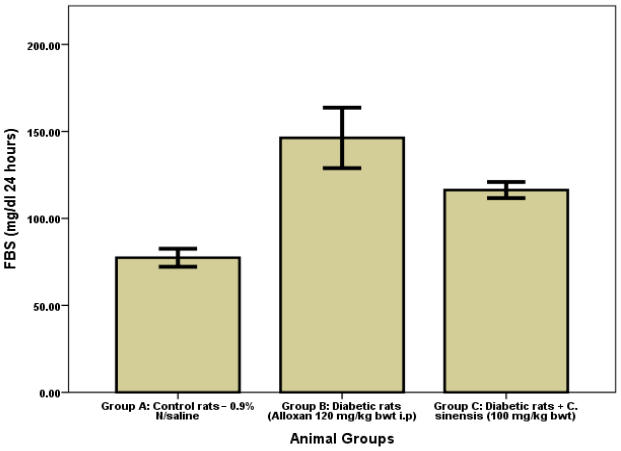

Figure 2. Bar graphs show fasting blood glucose levels in 3 rat groups compare with table II.

Discussion

Camellia sinensis has drawn much attention for its possible glucoregulatory and insulin secretory effects, providing a safe herbal option for diabetes mellitus.[13-16] Dietary fibers of plants are reported to reduce the diabetes mellitus incidence.[17] *Camellia sinensis* reportedly possess remarkable medicinal effects against various illnesses including hyperglycemia.[65] Anti – diabetic activity of *Camellia sinensis* has been demonstrated by previous studies[18-21] improved blood glucose, hyperglycemia and insulin in diabetic animal models.

Camellia sinensis is reported to exhibit glucoregulatory effects.[23] The present experimental study reveals similar glucose alleviating and insulin secretagogue effect of green tea. The findings of present study are consistent with previous studies[24,25] but contrary to other studies.[26,27]

Chandrakar Ankolekar et al,[27] explored effect of camellia sinensis and its polyphenolic metabolites extraction time with their therapeutic effect on hyperglycemia. This research highlights tea's polyphenol-rich benefits in managing early-stage type 2 diabetes. Comparing 2- and 5-minute extractions, longer steeping yielded higher phenolic content, stronger antioxidant activity, and greater α-glucosidase/α-amylase inhibition. Choice Darjeeling (5 minutes) was most effective. Findings suggest 5-minute extractions enhance tea's therapeutic potential for controlling postprandial hyperglycemia.

Firzan Nainu et al, [28] performed experimental study on Drosophilas. He first exposed the larvae to the high sucrose environment then exposed them with camellia sinensis. When flies on a high-sugar diet (causing hyperglycemia) were given C. sinensis extract, their survival improved. This benefit came from its ability to showing its potential role in managing hyperglycemia.

A.Gomes et al,[29] performed an experimental study on rats where he first induced hyperglycemia in rats by injecting streptozotocin in them and them fed them camellia sinensis for 4 weeks. Investigations were then carried out after completion of experimental period. Random blood glucose levels and fasting blood glucose levels before and after intake of camellia sinensis were compared and had proven the significant antihyperglycemic potential of Camellia sinensis.

Considering the evidences provided by previous studies and results of our current study it has shown that Camellia Sinensis is a potent antihyperglycemic agent.

Conclusion

In conclusion, the *Camellia sinensis* exerts significant glucose lowering in Alloxan induced male albino rat model. The findings are of clinical significance for treating hyperglycemia.

Recommendations: Human based studies with large sample size is required to validate the clinical findings of present study.

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