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Therapeutic Potential of Ethyl Acetate Fraction of Methanolic Extract of *Camellia Sinensis* (Green Tea) in Diabetes and Hyperlipidaemia Management in Streptozotocin induced Diabetic Male Albino Rat

Barnali Das[1, 2] and Debidas Ghosh[1]*

Molecular Medicine & Nutrigenomics Laboratory,
[1]Department of Bio-Medical Laboratory Science and Management,
Vidyasagar University, Midnapore, West Bengal, India -721 102.

[2] Department of Medical Laboratory Technology,
Debra Thana Sahid Kshudiram Smriti Mahavidyalaya, West Bengal, India- 721124.
***Corresponding author-** debidasghosh999@gmail.com

Abstract

This study investigates the therapeutic potential of the ethyl acetate fraction derived from the methanolic extract of *Camellia sinensis* (green tea) in managing diabetes and hyperlipidemia using a streptozotocin-induced diabetic male albino rat model. Diabetes and hyperlipidemia pose significant health challenges globally, with limited effective treatments available. Green tea, known for its rich polyphenolic content and antioxidant properties, has shown promising therapeutic effects in various metabolic disorders. In this research, streptozotocin-induced diabetic male albino rats were treated with the ethyl acetate fraction of green tea extract. Parameters such as blood glucose levels, lipid profile, and antioxidant enzymes activity were assessed. The findings reveal that treatment with the ethyl acetate fraction of methanolic extract of *Camellia sinensis* (green tea) resulted in significant ($p<0.05$) reductions in blood glucose levels and improvement in lipid profile parameters compared to the diabetic control group. Furthermore, histopathological examination indicated amelioration of pancreatic tissue damage in treated rats. These results suggest that the ethyl acetate fraction of green tea extract holds promise as a therapeutic agent for managing diabetes and hyperlipidemia. Further research elucidating the underlying mechanisms and clinical trials are warranted to validate its efficacy and safety for human use

Key words: *Camellia sinensis*, Diabetes, Carbohydrate metabolic enzymes, Hyperlipidemia, Transaminases enzymes.

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Introduction

Diabetes mellitus and hyperlipidemia are two interrelated metabolic disorders that have reached epidemic proportions globally, imposing significant health and economic burdens. Diabetes is characterized by hyperglycaemia resulting from insulin deficiency or resistance, while hyperlipidemia involves elevated levels of lipids in the bloodstream, particularly cholesterol and triglycerides. Both conditions are major risk factors for cardiovascular diseases, nephropathy, neuropathy, and retinopathy, contributing to morbidity and mortality worldwide **[Saeedi et al., 2019].** Current therapeutic approaches for diabetes and hyperlipidemia include lifestyle modifications, oral hypoglycaemic agents, insulin therapy, and lipid-lowering drugs. However, these interventions are often associated with adverse effects, limited efficacy, and challenges in achieving optimal glycaemic and lipid control [**ADA-2022**]. In recent years, there has been growing interest in exploring natural compounds with potential therapeutic effects on metabolic disorders. Among these, *Camellia sinensis*, commonly known as green tea, has garnered attention due to its rich polyphenolic content, including catechins, epicatechins, and flavonoids, which possess antioxidant, anti-inflammatory, and metabolic regulatory properties [**Yang et al., 2016**].

Numerous preclinical and clinical studies have suggested beneficial effects of green tea consumption on glucose metabolism, insulin sensitivity, lipid profile, and oxidative stress markers [**Bose et al., 2008**]. However, the bioactive components responsible for

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these effects and their mechanisms of action remain to be fully elucidated.

In this context, the present study aims to investigate the therapeutic potential of the ethyl acetate fraction derived from the ethyl-acetate fraction of methanolic extract of green tea for the management of diabetes and hyperlipidemia. Specifically, we focus on evaluating the effects of this fraction on blood glucose levels, lipid profile parameters, and histopathological changes in pancreatic tissue using a streptozotocin-induced diabetic male albino rat model.

By explaining the therapeutic effects of the ethyl acetate fraction of green tea extract on diabetes and hyperlipidemia, this research contributes to the growing body of evidence supporting the use of natural products as alternative or adjunctive therapies for metabolic disorders. Moreover, it lays the groundwork for further exploration of the underlying mechanisms and potential clinical applications of green tea-derived compounds in managing diabetes and hyperlipidemia.

Methods and Materials

Chemicals

Various chemicals and solvents utilized in this study were procured from reputable sources. These included products obtained from Sigma Chemical (St. Louis, MO, USA), Merck (KGaA, Germany), and other standard manufacturers. All chemicals were of analytical grade.

Preparation of fractionation from methanolic extract

The fresh green tea leaves of *Camellia sinensis* (green tea) from Subodh Brothers Pvt.

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Ltd. (Kolkata) were used in this study. The ethyl-acetate fraction of methanolic extract of green tea was prepared according to a standard protocol established by our laboratory **[Das et al., 2017]**.

Sampling of animals and their care

Twenty mature male rats of Wistar strain were obtained for this study. Upon arrival, the rats were acclimatized for a period of 7 days under standard environmental conditions. The rats were provided with standard laboratory feed and water *ad libitum* to ensure their well-being and nutritional needs were met.

Induction of Diabetes:

Adult 20 male rats of Wistar strain (80-90 days old) having body weight 140±10 g were used in this study. Fourteen of the acclimatized rats were subjected to diabetic induction using streptozotocin (STZ), following established protocols [**Das et al., 2017; Das et al., 2019**]. Fourteen rats made diabetic and out of these twelve rats fulfil the moderate diabetic criteria.

Ethical Approval:

The Institutional Ethic Committee (IEC) of Vidyasagar University gave the approval prior to conduct the experiment (IEC Approval No- IEC/3/C-5/14; Dated-3/11/14). The guidelines given by the CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals), Govt. of India, were strictly maintained.

Experimental design

The experimental animals were equally distributed into three groups, each comprising

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six animals. Diabetes was induced in two groups following established protocols [**Ali et al., 2009; Das et al., 2017**].

Group I: Vehicle control group: Animals in the control group were orally administered sterile water via gavage at a dosage of 2 ml per kg of body weight twice daily per rat as a vehicle treatment.

Group II: Vehicle diabetic group: Diabetic animals in this group received oral administrations of sterile water at a dosage of 2 ml per kg of body weight twice daily per rat.

Group III: Ethyl-acetate fraction treated diabetic group (10 mg/100 g body weight): Diabetic animals in this group were orally administered the ethyl acetate fraction at a dosage of 10 mg per 100 g of body weight in 2 ml of sterile water twice daily.

Treatment was continued for a duration of 28 days. On 29thday of treatment, experimental rats were sacrificed at fasting condition, according to guidelines provided by the CPCSEA.

Serum was separated for the estimation of cholesterol [**Abell et al., 1952**], triglyceride **[Fossati and Lorenzo, 1982],** high density lipoprotein cholesterol (HDLc) **[Warnick and Albers, 1978],** low density lipoprotein cholesterol (LDLc) and very low density lipoprotein cholesterol (VLDLc) [**Friedwald et al., 1972]**. The activities of hepatic hexokinase **[Chou and Wilson, 1975]** and glucose-6-phosphatase **[Swanson, 1955]** were measured. Antioxidative enzymes i.e. catalase (CAT) **[Beers and Sizer, 1952**], peroxidase (POD) **[Sadasivam and Manickam, 1996]** in liver was also measured.

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Glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) **[Henry et al., 1960]** in liver was also assessed.

Results

Fasting blood glucose level: Fasting blood glucose level was markedly elevated in the streptozotocin (STZ)-induced diabetic group compared to the corresponding vehicle-treated control group. Administration of the ethyl acetate fraction of Camellia sinensis for 28 days resulted in a significant reduction in fasting blood glucose levels compared to the STZ-induced diabetic group **(Table 1).**

Table 1: Recovery in fasting blood glucose level after treatment with ethyl-acetate fraction of *C. sinensis (*green tea) leaves in STZ-induced diabetic male albino rat

	Fasting blood glucose level (mg/dl)					
Groups	**(On the day of STZ injection)**	**1st day (The day of treatment started after 7days of injection)**	**7th day**	**14th day**	**21st day**	**29th day**
Vehicle treated control	**75.4±3.3[a]**	**74.3±3.4[a]**	**76.2±3.4[a]**	**78.8±3.4[a]**	**79.9±3.12[a]**	**76.7±3.2[a]**
Vehicle treated diabetic	**73.5±2.8[a]**	**330.4±3.4[b]**	**333.3±3.2[b]**	**330.6±3.3[b]**	**335.3±3.5[b]**	**336.6±3.4[b]**
Diabetic+ fraction treated	**73.4±3.2[a]**	**331.3±3.5[b]**	**285.3±3.4[c]**	**214.2±3.5[c]**	**163.3±4.4[c]**	**130.0±3.4[c]**

Each value represents mean ± SEM; n=6. ANOVA followed by multiple comparisons 'two tail t test'. In each vertical column, the mean value with different superscripts (a,b,c,) differ from each other significantly, $p<0.05$.

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Hepatic Hexokinase and Glucose-6-Phosphatase Activities: In the diabetic groups, hepatic hexokinase activity was decreased, while glucose-6-phosphatase activity was significantly increased compared to the corresponding vehicle-treated control group. Treatment with the ethyl acetate fraction of green tea for 28 days led to a notable increase in hexokinase activity and a significant decrease in glucose-6-phosphatase activity in diabetic animals **(Figure 1-2).**

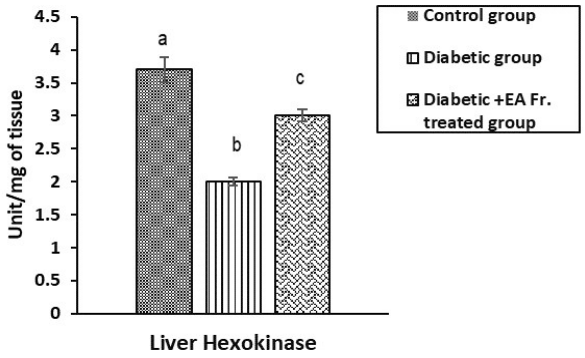

Fig. 1: Recovery in the activity of hexokinase in liver muscle after treatment of ethyl-acetate fraction of green tea in STZ-induced diabetic male albino rat: Bar expressed as Mean±S.E.M; n=6. ANOVA followed by multiple comparison 'two-tail t-test'. Bars with different superscripts (a,b,c) differ from each other significantly, $p<0.05$.

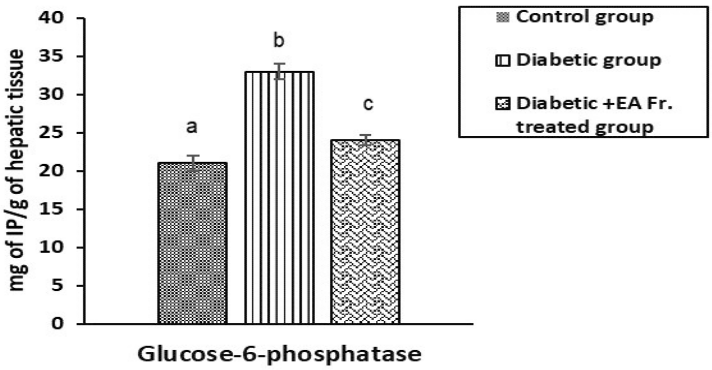

Fig. 2: Restoration in the activity of Glucose-6-phosphatase in liver muscle after administration with ethyl-

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acetate fraction of green tea in STZ-induced diabetic male albino rat: Bar expressed as Mean ±SEM; n=6. ANOVA followed by multiple comparison 'two-tail t-test'. Bars with different superscripts (a,b,c) differ from each other significantly, $p<0.05$.

Serum Lipid Profile: Serum VLDLc and LDLc levels were elevated, and HDLc levels decreased significantly in diabetic rats compared to controls. Administration of ethyl acetate fraction of green tea led to a significant decrease in VLDLc and LDLc levels, and a notable increase in HDLc levels. Additionally, total cholesterol and triglyceride levels decreased significantly after 28 days of fraction treatment in diabetic animals **(Figure 3-7).**

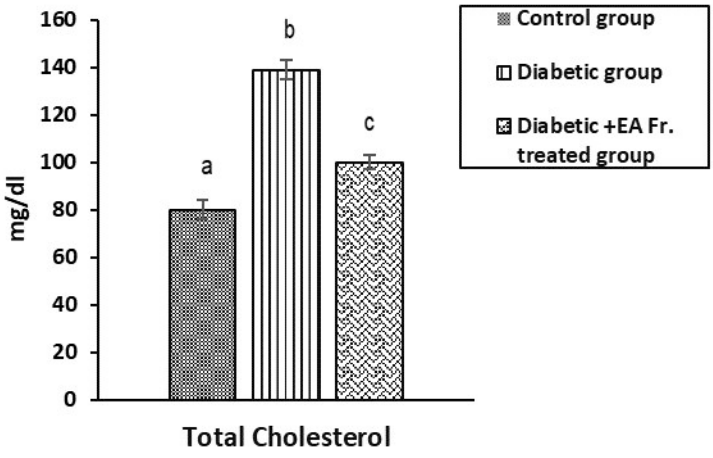

Fig. 3: The impact of the ethyl-acetate fraction of *C. sinensis* on serum total cholesterol in STZ-induced diabetic rats. Bar expressed as Mean ±SEM; n=6. ANOVA followed by multiple comparison 'two-tail t-test'. Bars with different superscripts (a,b,c) differ from each other significantly, $p<0.05$.

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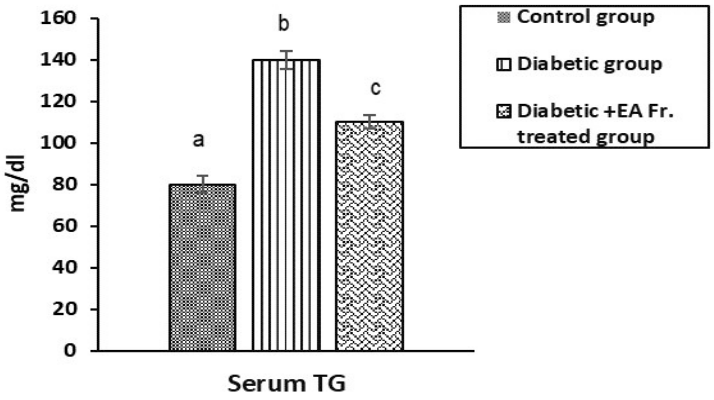

Fig. 4: The corrective efficacy of the ethyl-acetate fraction of *C. sinensis* on serum triglyceride (TG) level in STZ-induced diabetic rats. Bar expressed as Mean ±SEM; n=6. ANOVA followed by multiple comparison 'two-tail t-test'. Bars with different superscripts (a,b,c) differ from each other significantly, $p<0.05$

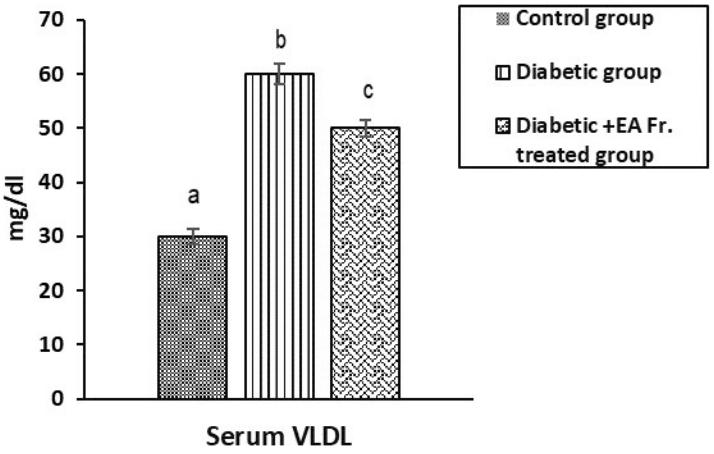

Fig. 5: Resettlement of serum VLDLc level following fraction treatment in STZ-induced diabetic male albino rats. Bar expressed as Mean±S.E.M; n=6. ANOVA followed by multiple comparison 'two-tail t test'. Bars with different superscripts (a,b,c) differ from each other significantly, $p<0.05$.

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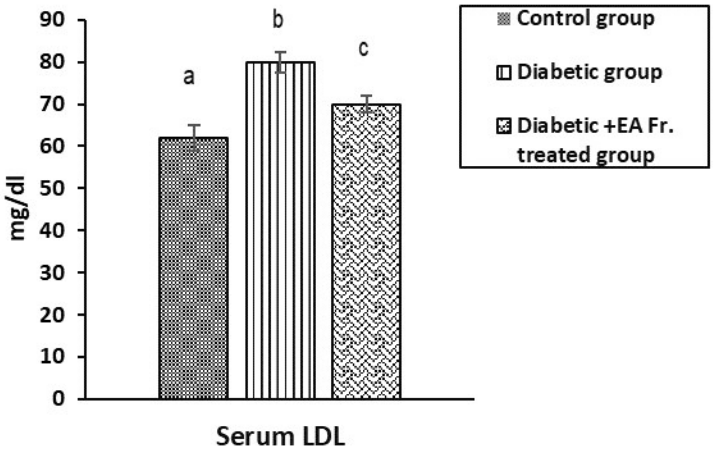

Fig. 6: Resettlement of serum VLDLc level following fraction treatment in STZ-induced diabetic male albino rats. Bar expressed as Mean±S.E.M; n=6. ANOVA followed by multiple comparison 'two-tail t test'. Bars with different superscripts (a,b,c) differ from each other significantly, $p<0.05$.

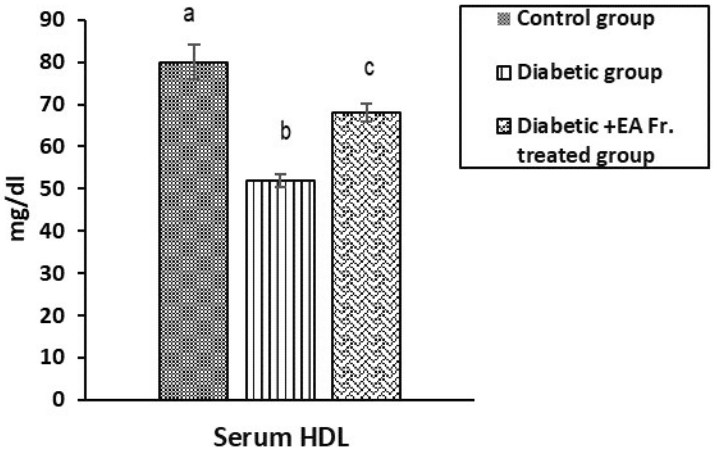

Fig. 7: Correction of serum HDLc level after administration of said fraction of C. sinensis in STZ-induced diabetic male albino rats. Bar expressed as Mean±S.E.M; n=6. ANOVA followed by multiple comparison 'two-tail t test'. Bars with different superscripts (a, b, c) differ from each other significantly, $p<0.05$.

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Catalase and Peroxidase Activities in Liver Muscle: Activities of peroxidase and catalase in the liver were significantly reduced in the diabetic group compared to the control group. Treatment with the ethyl acetate fraction of green tea for 28 days provided significant protection, leading to an elevation in the activities of these enzymes in liver tissue compared to the STZ-induced diabetic group **(Fig. 8-9).**

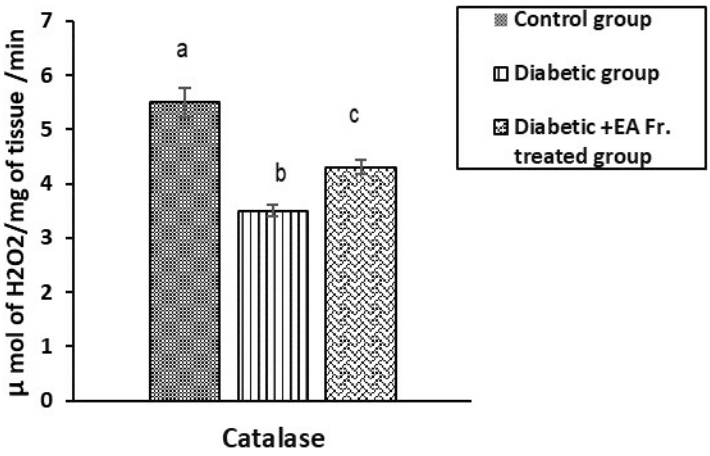

Fig. 8: Corrective consequence in the activity of catalase in liver after the treatment with ethyl-acetate fraction of *C. sinensis* in STZ-induced diabetic male albino rats. Bar expressed as Mean± S.E.M; n=6. ANOVA followed by multiple comparison 'two-tail t test'. Bars with different superscripts (a, b, c) differ from each other significantly, $p<0.05$.

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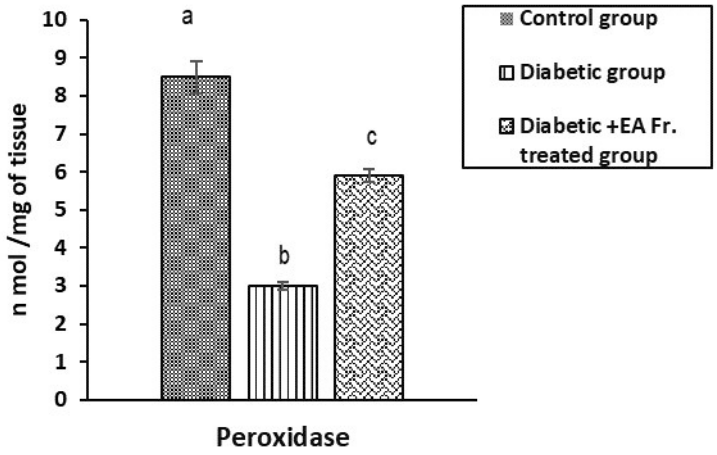

Fig. 9: Elevation in the activity of catalase in liver after the treatment with ethyl-acetate fraction of *C. sinensis* in STZ-induced diabetic male albino rats. Bar expressed as Mean±S.E.M; n=6. ANOVA followed by multiple comparison 'two-tail t test'. Bars with different superscripts (a, b, c) differ from each other significantly, $p<0.05$.

Liver Transaminase Activities: Activities of GOT and GPT in the liver were significantly elevated in the diabetic rats compared. After the treatment with the said fraction of green tea resulted in a significant decrease in GOT and GPT activities, in respect to STZ treated diabetic group **(Fig. 10).**

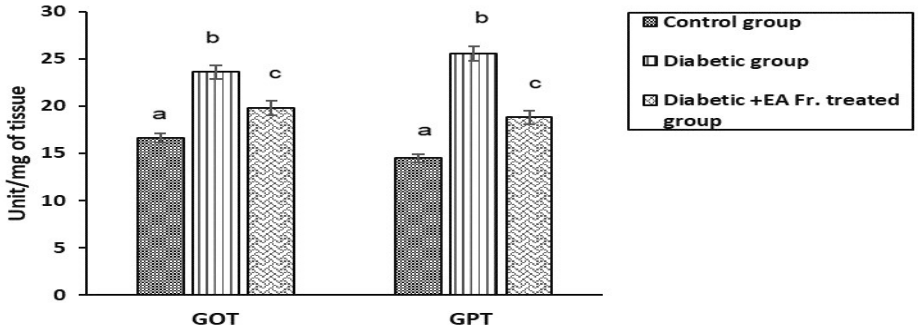

Fig. 10: Efficiency of ethyl-acetate fraction of *C. sinensis* treatment on the activities of hepatic GOT, GPT in streptozotocin-induced diabetic male albino rats. Data represents Mean ± SEM (n = 6). ANOVA followed by multiple comparison 'two-tail t test'. Bars

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with different superscripts (a, b, c) differ from each other significantly, $p<0.05$.

Discussion

The global burden of diabetes is rapidly escalating, necessitating effective treatment strategies to address this growing public health challenge **[Baydas et al., 2003; Saeedi et al., 2019]**. *Camellia sinensis* (green tea), has gained popularity worldwide as a beverage with various health benefits. In this context, our experiment was designed to investigate the therapeutic potential of the ethyl acetate fraction of the methanolic extract of *Camellia sinensis* in managing diabetes and hyperlipidemia in streptozotocin-induced diabetic male albino rats.

Our findings emphasize the importance of exploring natural compounds like green tea for diabetes management. The ethyl acetate fraction of methanolic green tea extract demonstrated promising effects in ameliorating key metabolic parameters associated with diabetes and hyperlipidemia. Consistent with previous studies, STZ-induced diabetic rats exhibited markedly elevated fasting blood glucose levels compared to the vehicle-treated control group [**Ali et al., 2009; Das et al., 2017**]. This hyperglycaemic state is characteristic of diabetes mellitus and underscores the severity of the diabetic condition in our experimental model. The significant reduction in fasting blood glucose levels observed following treatment with the ethyl acetate fraction suggests its potential as a hypoglycaemic agent. This effect could be attributed to the presence of bioactive compounds such as catechins and flavonoids in green tea, which have been reported to enhance insulin sensitivity and improve glucose metabolism **[Wen et al., 2022].** Hepatic

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hexokinase activity, an enzyme involved in glucose metabolism, was found to be decreased in diabetic rats, while glucose-6-phosphatase activity, responsible for gluconeogenesis, was significantly increased compared to the control group. These alterations in enzyme activities reflect dysregulated hepatic glucose metabolism in diabetes. Treatment with the ethyl acetate fraction of green tea restored hexokinase activity and attenuated glucose-6-phosphatase activity, indicating improved hepatic glucose utilization and reduced gluconeogenesis in diabetic animals. These findings suggest that the green tea fraction may exert its hypoglycaemic effects through modulation of hepatic glucose metabolism pathways.

Dyslipidaemia is a common metabolic complication associated with diabetes, characterized by elevated levels of triglycerides, total cholesterol, LDL cholesterol, and decreased levels of HDL cholesterol **[Ali et al., 2009; Schofield et al., 2016].** Consistent with this, STZ-induced diabetic rats exhibited elevated serum levels of VLDL cholesterol and LDL cholesterol, along with decreased HDL cholesterol levels compared to the control group. However, treatment with the ethyl acetate fraction of green tea resulted in a significant improvement in the serum lipid profile, with reduced levels of VLDL cholesterol and LDL cholesterol, and increased levels of HDL cholesterol after 28 days of administration. Additionally, significant reductions in total cholesterol and triglyceride levels were observed, indicating the potential lipid-lowering effects of the green tea fraction in diabetic conditions. Oxidative stress plays a crucial role in the pathogenesis of diabetes and its complications [**Halliwell and Gutteridge, 2007;**

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Pandey and Rizvi, 2010; Biswas et al., 2017].. In our study, diabetic rats exhibited reduced activities of hepatic catalase and peroxidase enzymes, indicative of impaired antioxidant defence mechanisms. However, treatment with the ethyl acetate fraction of green tea significantly elevated the activities of these antioxidant enzymes in liver tissue, suggesting enhanced protection against oxidative damage. These findings suggest that the green tea fraction may exert antioxidative effects, thereby mitigating oxidative stress associated with diabetes.

Elevated levels of liver transaminases, such as GOT and GPT, are indicative of liver injury and dysfunction, commonly observed in diabetic conditions [**Mallick et al., 2007]**. Our results demonstrate that treatment with the ethyl acetate fraction of green tea significantly reduced the activities of GOT and GPT in diabetic rats, approaching levels observed in the control group. These findings suggest potential hepatoprotective effects of the green tea fraction, leading to the restoration of liver function in diabetic animals.

Conclusion

In conclusion, our study provides evidence supporting the therapeutic potential of the ethyl acetate fraction of *Camellia sinensis* (green tea) in managing diabetes and associated metabolic abnormalities in streptozotocin-induced diabetic rats. Our findings suggest that the ethyl acetate fraction of *Camellia sinensis* holds promise as a complementary therapeutic agent for diabetes and hyperlipidemia management.

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[Scheme Code: 172/ 2014] to conduct this work is gratefully acknowledged.

Conflict of interest

We have no conflict of interest.

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