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# Elucidation of Antidiabetic Mechanism of *Centella asiatica* and *Zingiber officinale*- An *In-vitro* and *In-vivo* approach

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**ABSTRACT**: *Zingiber officinale* rhizome and *Centella asiatica* leaves are extensively consumed as dietary supplements, and they are also reported for their antidiabetic activity. However, no proper scientific evidence exists to support its antidiabetic property at a mechanistic level. The present study was designed to assess the *in-vitro* and *in-vivo* antidiabetic activity of hydroalcoholic extracts of *Zingiber officinale* rhizome and *Centella asiatica* leaves. *In-vitro* assays like alpha amylase as well as alpha glucosidase inhibition assay and glucose uptake by yeast cell assay have been performed. *In-vivo* activity was also evaluated in streptozotocin-induced diabetic albino rats. Acarbose and metformin are taken as standard drugs for comparison. Both the extracts elicited significant antidiabetic activity both *in-vitro* and *in-vivo*. The suppression of alpha amylase and alpha glucosidase enzymes is connected with their anti-diabetic action. It also demonstrated its impact by increasing glucose absorption by peripheral tissue and may have insulin memetic action. Although *Centella asiatica* was slightly more active than Ginger. It is very likely that regular ingestion of these two herbs in conjunction with conventional hypoglycemic medicines will aid in the maintenance of blood glucose levels in diabetic patients.

**KEYWORDS**: Albino rats; *Centella asiatica*; Diabetes mellitus; *In-vitro*; *In-vivo*; Streptozotocin; *Zingiber officinale*.

## 1. INTRODUCTION

Diabetes mellitus (DM) is a metabolic condition characterised by problems in the metabolism of carbohydrates, proteins, and lipids. It occurs as a result of either a shortage or lack of insulin secretion (T1DM) or a diminished sensitivity of the tissues to insulin (T2DM), and can result in persistent hyperglycemia. Uncontrolled diabetes can lead to a variety of problems or secondary disorders. According to the World Health Organization (WHO), there were 171 million diabetes diagnoses globally in 2000, with that figure anticipated to rise to 366 million by 2030 [1]. Diabetes mellitus causes infection susceptibility, neuropathy, nephropathy, retinopathy, angiopathy, hyperlipidemia, and ketoacidosis. These factors contribute to increased disability and lower life spans [2]. T2DM affects around 460 million individuals worldwide, accounting for almost 90% of the population. According to statistics, in 25 years, that number will have risen to over 700 million [3].

Traditional and folk medicine play an essential role in global health care. Because of their safety, herbal medicines are used and acknowledged all over the world. A vast number of Indian pharmaceutical companies are currently creating natural drugs [4]. *Centella asiatica* (CA) (Umbelliferae family) is a popular Indian herb. It has traditionally been used to treat a variety of CNS illnesses, including epilepsy, schizophrenia, and cognitive impairment. It is also used to cure diseases such as leprosy, asthma, jaundice, diarrhoea, hepatitis, syphilis, and smallpox. It is also anti-inflammatory, antipyretic, and analgesic [5,6]. Ginger is an underground rhizome of the plant *Zingiber officinale Roscoe*, which belongs to the Zingiberaceae family [7]. It is a dietary supplement used to cure a variety of ailments such as cough, asthma, anorexia, indigestion, and constipation. [8].

Both ginger rhizome and *C. asiatica* leaves are popular nutritional ingredients. Several investigations have shown that it has modest anti-diabetic action. Routine consumption of these herbs with adequate

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information may assist in managing diabetes mellitus and its associated risks through various mechanisms. However, adequate scientific evidence to explore their mechanisms for antidiabetic action is lacking. Hence, the Present study aims to elucidate the antidiabetic activity of hydroalcoholic extracts of *Zingiber officinale* rhizome and *Centella asiatica* leaf with a possible mechanism of action.

## 2. RESULTS

### 2.1. *In-vitro* antidiabetic activity

2.1.1. Alpha amylase inhibitory assay

*Centella asiatica* leaf extract (CAs), *Zingiber officinale* rhizome extract (ZO) and acarbose have been tested *invitro* for Alpha amylase inhibition activity. Both the extracts showed significant dose-dependent inhibitory activity. Significantly higher inhibitory activity is observed in Acarbose followed by CAs and ZO as shown in **Table 1 and Figure 1**. The IC50 value of ascorbic acid, CAs and ZO was found to be 148.94, 695.57 and 1084.90 respectively.

**Table 1.** Inhibition of alpha amylase enzyme assay

| Sl. No. | Drug | Concentration (µg/mL) | UV Absorbance at 540 nm | % Inhibition | $IC_{50}$ Value |
|---|---|---|---|---|---|
| **1** | Acarbose | 50 | 0.064±0.001 | 28.36±1.119** | 148.94 |
| | | 100 | 0.052±0.001 | 41.42±1.709** | |
| | | 200 | 0.032±0.001 | 64.18±1.119*** | |
| | | 400 | 0.028±0.001 | 68.28±1.292*** | |
| | | 800 | 0.019±0.001 | 77.99±1.292*** | |
| | | 1600 | 0.018±0.001 | 79.85±1.119*** | |
| **2** | CAs | 50 | 0.079±0.002 | 11.57±2.238* | 695.57 |
| | | 100 | 0.073±0.003 | 18.28±3.358* | |
| | | 200 | 0.066±0.001 | 25.75±1.292** | |
| | | 400 | 0.057±0.001 | 36.19±1.119** | |
| | | 800 | 0.041±0.001 | 54.10±1.119*** | |
| | | 1600 | 0.032±0.002 | 64.18±2.961** | |
| **3** | ZO | 50 | 0.081±0.001 | 9.33±1.119* | 1084.90 |
| | | 100 | 0.076±0.002 | 14.55±2.330* | |
| | | 200 | 0.067±0.00 | 24.25±1.709** | |
| | | 400 | 0.057±0.000 | 35.82±0.646*** | |
| | | 800 | 0.044±0.001 | 50.75±1.938** | |
| | | 1600 | 0.036±0.001 | 59.70±1.119*** | |

Values are expressed as the means of three replicates ± Standard Deviation (SD), n=3, Statistically significant ($P < 0.05$), ***$P<0.001$, **$P< 0.01$ and *$P< 0.05$ (vs. control)

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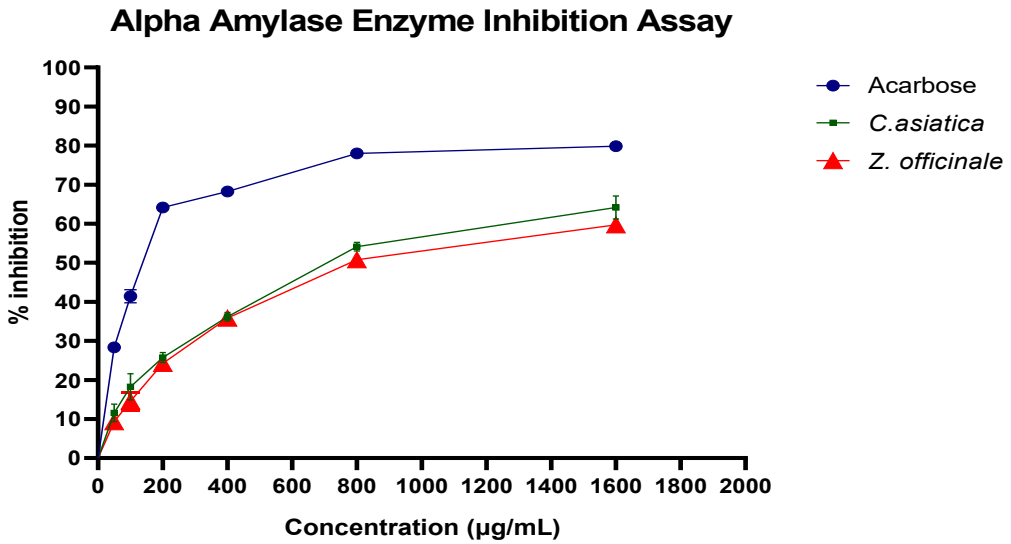


**Figure 1.** Inhibition of alpha amylase enzyme assay

2.1.2. Alpha glucosidase inhibitory assay

CAs, ZO and standard drug Acarbose has been tested *invitro* for Alpha amylase inhibition activity. All the samples showed significant dose-dependent inhibitory activity. Significantly higher inhibitory activity is observed in Acarbose than in ZO and CAs as shown in **Table 2 and Figure 2**. The IC50 value of ascorbic acid, CAs and ZO was found to be 143.89, 718.71 and 675.20 respectively.

**Table 2**. Inhibition of alpha glucosidase enzyme assay

| Sl. No. | Drug | Concentration (µg/mL) | UV Absorbance at 505 nm | % Inhibition | $IC_{50}$ Value |
|---|---|---|---|---|---|
| **1** | Acarbose | 50 | 0.34±0.01 | 36.02±1.075** | 143.89 |
| | | 100 | 0.3±0.01 | 44.10±1.863** | |
| | | 200 | 0.23±0.01 | 56.52±1.075*** | |
| | | 400 | 0.18±0.01 | 65.84±1.075*** | |
| | | 800 | 0.10±0.01 | 80.75±1.075*** | |
| | | 1600 | 0.05±0.01 | 89.44±2.151*** | |
| **2** | CAs | 50 | 0.483±0.02 | 9.94±3.878$^{ns}$ | 718.71 |
| | | 100 | 0.45±0.02 | 16.15±3.726 $^{ns}$ | |
| | | 200 | 0.4±0.01 | 25.47±1.863** | |
| | | 400 | 0.326±0.01 | 39.13±2.15** | |
| | | 800 | 0.263±0.01 | 50.93±2.151** | |
| | | 1600 | 0.183±0.02 | 65.84±65.84** | |
| **3** | ZO | 50 | 0.476±0.03 | 11.18±5.692 $^{ns}$ | 675.20 |
| | | 100 | 0.43±0.01 | 19.88±1.863* | |
| | | 200 | 0.39±0.01 | 27.33±1.863** | |
| | | 400 | 0.316±0.01 | 40.99±2.151** | |
| | | 800 | 0.25±0.01 | 53.42±1.863** | |
| | | 1600 | 0.173±0.02 | 67.70±2.846** | |

Values are expressed as the means of three replicates ± Standard Deviation (SD), n=3, Statistically significant (P < 0.05), ***P<0.001, **P< 0.01 and *P< 0.05 (vs. control)

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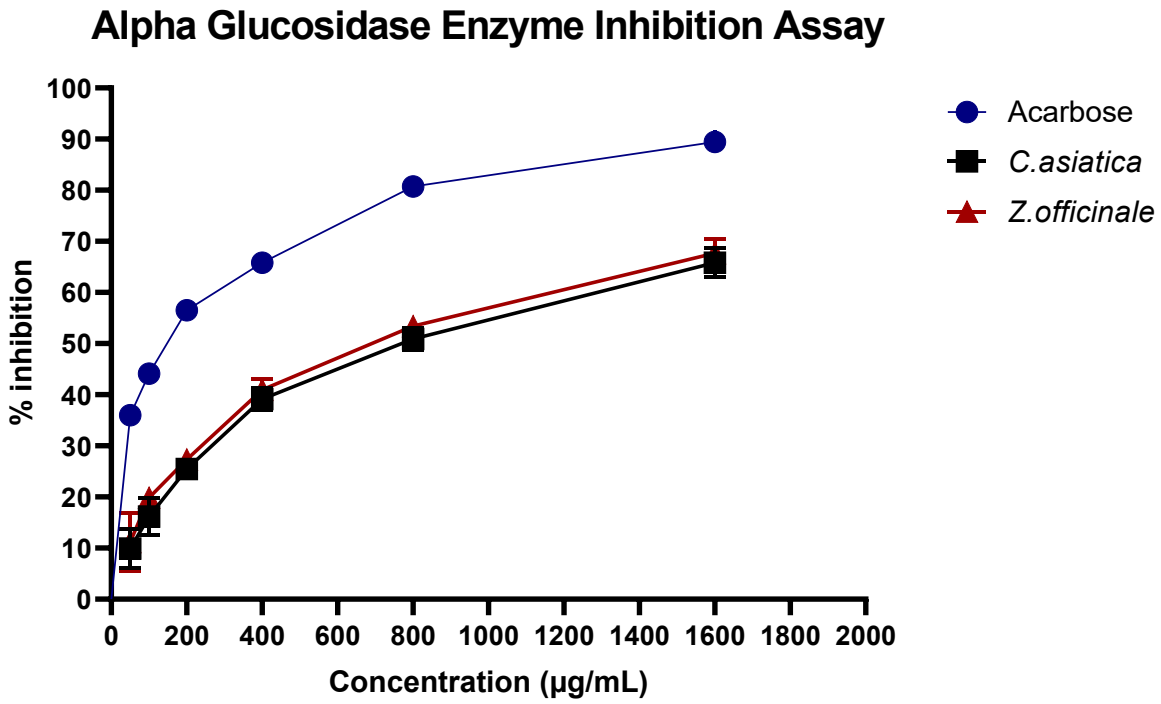


**Figure 2**. Inhibition of alpha glucosidase enzyme assay

2.1.3. Glucose Uptake Assay by Yeast Cells

The rate of glucose transport across the cell membrane in the yeast cell system at 5mM glucose concentration is presented in **Table 3** and **Figure 3**. Yeast cells were treated with CAs, ZO and standard drug metformin at a concentration of 50 µg to 1600 µg and glucose uptake activity was observed.

The percentage of glucose uptake by yeast cells has been enhanced after treatment. Both the extracts showed dose-dependent increase in glucose uptake. CAs showed higher activity as compared to ZO.

**Table 3.** Determination of Glucose Uptake Assay by Yeast Cells in 5 mM Glucose Concentration

| Sl. No. | Drug | Concentration (µg/mL) | Absorbance at 520 nm | % Increase in Glucose Uptake |
|---|---|---|---|---|
| 1 | Metformin | 50 | 0.117±0.003 | 42.389±1.578** |
| | | 100 | 0.103±0.001 | 49.099±0.750*** |
| | | 200 | 0.086±0.002 | 57.446±1.022*** |
| | | 400 | 0.068±0.002 | 66.612±1.299*** |
| | | 800 | 0.041±0.003 | 79.541±1.500*** |
| | | 1600 | 0.022±0003 | 89.198±1.472*** |
| 2 | CAs | 50 | 0.154±0.000 | 24.058±0.283*** |
| | | 100 | 0.140±0.001 | 30.932±0.566*** |
| | | 200 | 0.125±0.001 | 38.625±0.490*** |
| | | 400 | 0.104±0.000 | 48.445±0.283*** |
| | | 800 | 0.094±0.0005 | 53.682±0.283*** |
| | | 1600 | 0.066±0.002 | 67.594±1.299*** |
| 4 | ZO | 50 | 0.156±0.001 | 23.076±0.750** |
| | | 100 | 0.136±0.002 | 33.224±0.981** |
| | | 200 | 0.124±0.002 | 38.952±1.022** |
| | | 400 | 0.102±0.001 | 48.936±0.750*** |
| | | 800 | 0.097±0.002 | 52.209±1.022*** |
| | | 1600 | 0.072±0.002 | 64.320±1.022*** |

Values are expressed as the means of three replicates ± Standard Deviation (SD), n=3,
Statistically significant (P < 0.05), ***P<0.001, **P< 0.01 and *P< 0.05 (vs. control)

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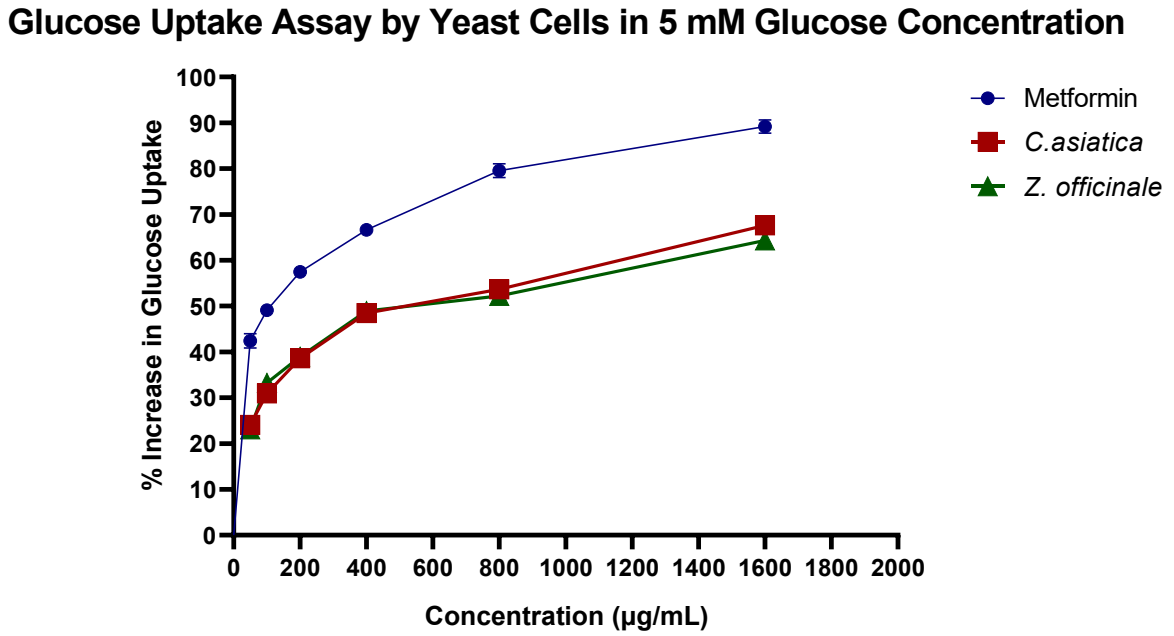


**Figure 3.** Glucose Uptake Assay by Yeast Cells in 5 mM Glucose Concentration

### 2.2. *In-vivo* antidiabetic activity

2.2.1. Blood Glucose Level

Effect of CAs, ZO and metformin on blood sugar level in streptozotocin induced diabetic rats are depicted in the **Table 4 and Figure 4**. Blood glucose was measured in normal and diabetic rats on the 1st, 7th, 14th and 21st days of treatment. A significantly elevated glucose level is observed in untreated diabetic rats as compared to normal rats. Oral administration of CAs and ZO at 200 mg/ kg b.w and the standard drug metformin at 90 mg/kg b.w showed a significant reduction in blood glucose level ($P < 0.05$) in dose-dependent and time-dependent manner except for ZO on the 1st day of treatment. CAs showed slightly higher activity as compared to ZO.

**Table 4**. Effect of CAs and ZO on blood glucose level in STZ-induced diabetic rats

| Group | Blood glucose levels (mg/dl) (Mean ± SEM) | | | |
|---|---|---|---|---|
| | **1st day** | **7th day** | **14th day** | **21st day** |
| **Group I (Normal control)** | 103.83±1.62 | 103.66±0.76 | 102.00±0.96 | 103.00±1.06 |
| **"Group II (Diabetic control)** | 366.66±4.60 | 368.83±9.53 | 378.33±9.22 | 386.66±8.89 |
| **Group III (CAs 200)** | 341.33±3.80* | 301.33±1.80*** | 280.83±3.94*** | 261.83±3.09*** |
| **Group IV (ZO 200)** | 362.33±3.97 ns | 325.33±2.80*** | 297.33±1.87*** | 277.16±1.57*** |
| **Group V (Metformin)** | 350.83±1.74* | 255.50±1.17*** | 173.83±1.49*** | 105.33±1.49*** |

Values are expressed as mean ± SEM, (n=6); P<0.05(*), P<0.01(**), P<0.001(***) compared to diabetic animals (two-way ANOVA followed by a Dunnett's t-test). P-values <0.05 were considered statistically significant.

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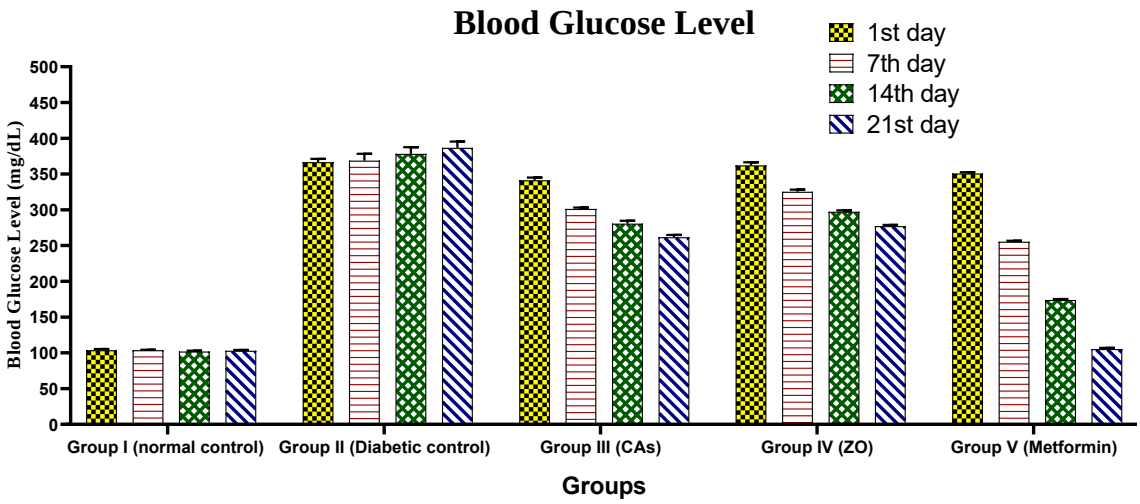


**Figure 4**. Effect of CAs and ZO on blood glucose level in STZ-induced diabetic rats.

2.2.2. Effect on Lipid Profile

The effect of CAs, ZO, and metformin on the lipid profile in streptozotocin-induced diabetic rats are shown in **Table 5 and Figure 5**. A significant elevated level of TC, TG, LDL, VLDL and reduced level of HDL have been observed in the diabetic control group of rats on the 21st day. Such changes in lipid profile were avoided in treatment groups and brought closer to normal dose dependently. Efficient results were observed in Metformin, followed by CAs and Z.O.

**Table 5.** Effect of CAs and ZO on lipid profile in STZ-induced diabetic rats.

| Group | Lipid Profile (mg/dL) | | | | |
|---|---|---|---|---|---|
| | TC | TG | HDL | LDL | VLDL |
| **Group I (Normal control)** | 65.33±1.54*** | 87.66±1.70*** | 38±0.36*** | 9.8±1.17*** | 17.53±0.34*** |
| **"Group II (Diabetic control)** | 132.83±1.35*** | 191.66±2.01*** | 17.16±1.19*** | 77.33±1.22*** | 38.33±0.40*** |
| **Group III (CAs 200)** | 103±1.15*** | 153.5±1.83*** | 28.66±0.66*** | 43.63±1.60*** | 30.7±0.36*** |
| **Group IV (ZO 200)** | 105.16±2.02*** | 157.5±1.11*** | 26.16±0.47*** | 47.5±2.11*** | 31.5±0.22*** |
| **Group V (Metformin)** | 69.5±0.99*** | 98.33±0.88*** | 34.16±0.79*** | 15.66±1.00*** | 19.66±0.17*** |

Values are expressed as mean ± SEM, (n=6); P<0.05(*), P<0.01(**), P<0.001(***) compared to diabetic animals (two-way ANOVA followed by a Dunnett's t-test). P-values <0.05 were considered statistically significant.

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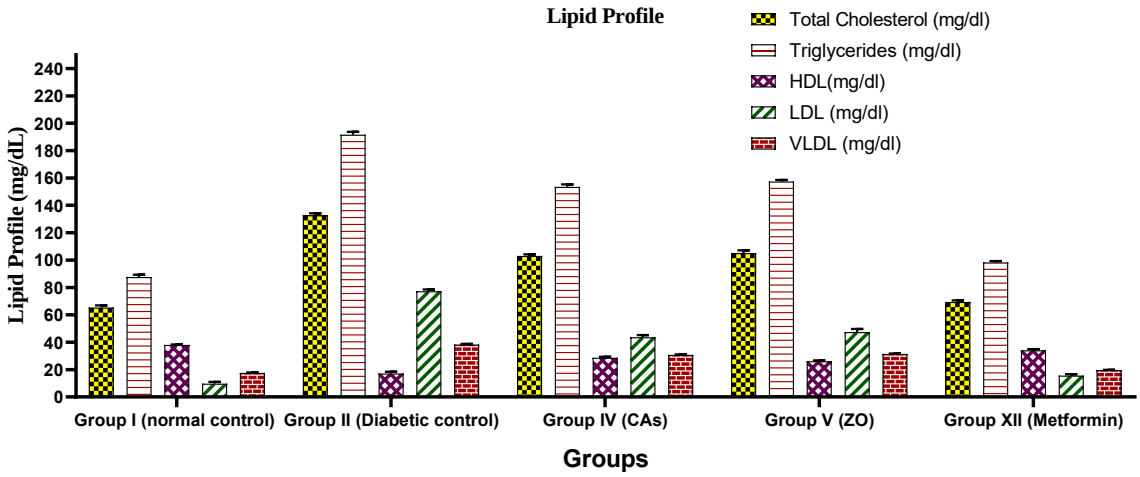
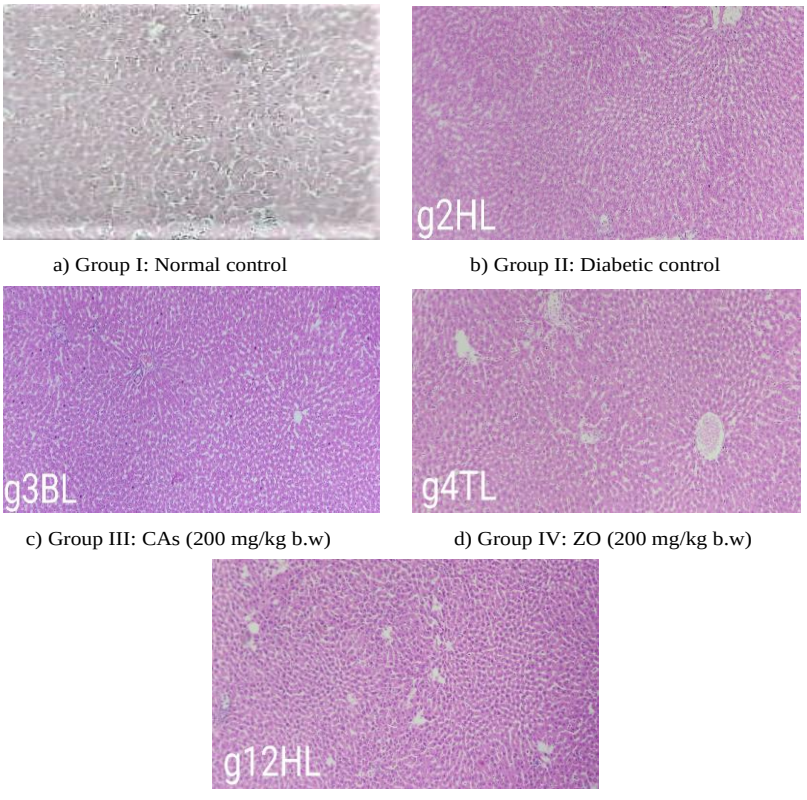



**Figure 5.** Effect of CAs and ZO on lipid profile in STZ-induced diabetic rats.

2.2.3. Histopathological Examination (Liver cells)

The liver histoarchitecture was appropriate in the normal control rats with normal Kupffer cell (KC) distribution, sinusoids and hepatic parenchyma. The liver of the diabetic control rat revealed necrotic changes, dilatation of liver sinusoids (LS), activation of KC, and cytoplasmic vacuolization (CV) of hepatocytes. Post-treatment of diabetic rats with conventional and test medications resulted in variable degrees of improvement. The liver of the metformin-treated group showed mild degenerative changes and dilatation of the hepatic sinusoid with a relatively higher number of KC. The liver histoarchitecture of the CAs and ZO treated group was more or less regular, with relatively minor necrobiotic changes and degeneration (**Figure 6**).




a) Group I: Normal control
b) Group II: Diabetic control
c) Group III: CAs (200 mg/kg b.w)
d) Group IV: ZO (200 mg/kg b.w)
e) Group V: Metformin (90 mg/kg b.w)

**Figure 6.** The liver section of histology stained with hematoxylin and eosin (Scale bar 50 µm, 100x magnification).
a) Group I: Normal control, b) Group II: Diabetic control (received STZ), c) Group III: CAs (200 mg/kg b.w), d) Group IV: ZO (200 mg/kg b.w), e) Group V: Metformin (90 mg/kg b.w).

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2.2.4. Histopathological Examination (Pancreatic cells)

The histological architecture of the pancreas was typical in normal control rats. It took the form of an acinar structure with normal Langerhans islets. The pancreas of the diabetic control group showed a considerable loss in islets of Langerhans (IL) as well as acini atrophy, vacuolar degeneration, and necroptosis. Improvements in pancreatic cells were observed in both the standard and test drug-treated groups. IL returned to normal size in the metformin-treated group, with normal acinar cells and mild necrotic changes. The pancreas of the CAs and ZO-treated groups showed a moderate restoration of IL cells with little necrotic changes. With the restoration of IL and minimal necrotic changes, the pancreas of the extract-treated group seemed more or less normal (**Figure 7**).

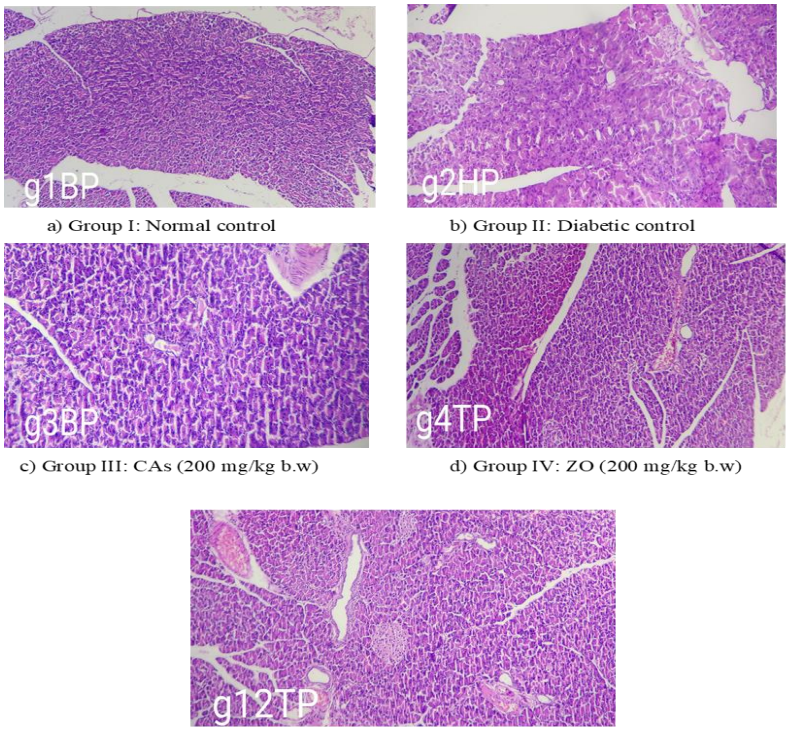


**Figure 7.** The Pancreatic section of histology stained with hematoxylin and eosin (Scale bar 50 µm, 100x magnification). a) Group I: Normal control, b) Group II: Diabetic control (received STZ), c) Group III: CAs (200 mg/kg b.w), d) Group IV: ZO (200 mg/kg b.w), e) Group V: Metformin (90 mg/kg b.w).

## 3. DISCUSSION

In this study, the antidiabetic activity of hydroalcoholic extracts of *Centella asiatica* leaf and *Zingiber officinale* rhizome has been evaluated by *in-vitro* and *in-vivo* methods. *In-vitro* methods such as alpha amylase inhibition assay, alpha glucosidase inhibition assay, glucose absorption by yeast cell have been performed. *In-vivo* antidiabetic activity of both the extracts was performed in streptozotocin-induced diabetic rats.

A variety of *in-vitro* assays can be used to assess a drug's anti-diabetic potential, providing insight into its *in-vivo* activity. One of the primary enzymes that hydrolyze starch into smaller oligosaccharides is pancreatic-amylase. By the action of alpha glucosidase, these oligosaccharides are digested to glucose and taken into the bloodstream. As a result, blood glucose levels rose. Inhibiting alpha amylase and glucosidase enzymes slows carbohydrate digestion and glucose absorption into the bloodstream. As a result, inhibiting these enzymes may be advantageous in the treatment of type 2 diabetes [15].

The colorimetric approach is used in both amylase and glucosidase inhibitory assays. Amylase is an enzyme that transforms starch to maltose. The amount of maltose produced is determined by reducing 3,5 dinitrosalisylic acid (3,5 DNSA). Maltose turns alkaline 3,5 DNSA from a pale yellow to an orange-red tint. The color's intensity is proportional to the amount of maltose in the sample. At 540 nm, the intensity of colour change is determined using a spectrophotometer [16]. Glucose oxidase (GOD) turns glucose to gluconate and hydrogen peroxide (H2O2) in the glucosidase inhibitory experiment. In the presence of peroxidase (POD), hydrogen peroxide oxidises the chromogen (4-aminophenazone + phenol) to produce a

red or pink coloured molecule. At 505 nm, the intensity of the red/pink colour is related to the content of glucose. [17, 18]

CAs and ZO have also been tested for anti-diabetic efficacy using *Saccharomyces cerevisiae* (Baker's yeast). The yeast cell's ability to absorb glucose is one of the characteristics that determines its anti-diabetic effectiveness. The process of facilitated diffusion may be involved in the transport of glucose throughout the yeast cell. [19]. According to several studies, *Saccharomyces cerevisiae* regulates the expression of glucose transporter genes. Snf3 and Rgt2 are integral plasma membrane proteins with distinct carboxy-terminal domains that are predicted to be found in the cytoplasm. The cytoplasmic domains of Snf3 and Rgt2 act as glucose receptors and are essential for the transmission of a glucose signal [20].

To execute *in-vivo* activity, a streptozotocin (STZ) induced diabetes model was used. Streptozotocin is derived from *Streptomycetes achromogenes* and is used to cause type I and type II diabetes. STZ enters the beta cell by a glucose transporter (GLUT-2) and causes alkylation of DNA, which results in the release of hazardous levels of nitric oxide (NO), which limits aconitase activity and contributes to DNA damage. Beta cells are destroyed by necrosis as a result of STZ activity. [21].

*In-vitro* assays were performed with different concentrations of CAs and ZO ranging from 50 to 1600 µg/mL. Results of the *in-vitro* and *in-vivo* activities confirmed that both CAs and ZO have potential antidiabetic activity in a dose-dependent manner. Inhibition of alpha amylase and absorption of glucose by the yeast cell seems better in CAs than in ZO, at the same time alpha glucosidase inhibition is quite better in ZO. In streptozotocin induced diabetic rats both the extract at a dose of 200 mg/kg bodyweight showed significant hypoglycemic activity by reducing blood glucose level.

Extracts also displayed marked antihyperlipidemic activity by reducing TC, TG, LDL, VLDL and increasing HDL. Both Antihyperglycemic and Antihyperlipidemic activity are quite effective in CAs as compared to ZO. *In-vitro* and *In-vivo* studies indicated that the mechanism behind the antidiabetic activity of these extracts is the inhibition of alpha amylase, alpha glucosidase enzyme and increase in the absorption of glucose by peripheral tissue. It may also be due to its insulin memetic action.

## 4. CONCLUSION

Both *Centella asiatica* and *Zingiber officinale* demonstrated significant antidiabetic activity by suppressing alpha amylase and alpha glucosidase enzymes. It also demonstrated its action by increasing glucose absorption by peripheral tissue, and it may have insulin memetic action. Although *Centella asiatica* demonstrated greater activity than Ginger, it is likely that regular consumption of these two herbs in conjunction with conventional hypoglycemic agents will help diabetic patients maintain blood glucose levels and reduce secondary complications of diabetes. These herbs can be formulated to a suitable dosage form for diabetic patients.

## 5. MATERIALS AND METHODS

### 5.1. Chemicals and reagents

Porcine pancreatic α-amylase, rat intestinal α-glucosidase, glucose oxidase, peroxidase reagent, acarbose, metformin and streptozotocin were obtained from Yarrow Chem Products, Mumbai, Maharashtra, India. Starch, sucrose and dinitrosalicylic acid (DNS) were purchased from Sisco Research Laboratories Pvt. Ltd. Taloja, Maharashtra, India. The remaining chemicals and reagents were of analytical grade.

### 5.2. Plant material

In the current study, leaves of *Centella asiatica* and rhizomes of *Zingiber officinale* were collected in Nagamangala Taluk, Mandya District, Karnataka. Plant parts were authenticated in the herbarium centre, Foundation for Revitalisation of Local Health Traditions, Bangalore, India (FRLHT Acc. No. of plants is 5551 and 5553 for *Centella asiatica* and *Zingiber officinale*, respectively). To eliminate any contamination, the plant's components underwent a thorough cleaning. After nearly a week of drying in the shade, the parts were ground to a coarse powder. Powders were stored for subsequent use in an airtight container and in a hygienic location.

### 5.3. Preparation of Extract

The dried powder of *Centella asiatica* leaves, and *Zingiber officinale* rhizome were extracted with 70% ethanol by cold maceration method [9, 10].

**5.4. *In-vitro* antidiabetic assay**

5.4.1. Alpha amylase inhibitory assay

1mg/ml of enzyme, test samples and standard drug (Acarbose) was prepared using Sodium Phosphate buffer (0.2 M PH 4.5). In 1.5 mL microcentrifuge tubes, 10 L Alpha amylase enzyme and test samples or standard medication (Acarbose) at varied concentrations ranging from 50 to 800 µg/mL were added and incubated at room temperature for 20 minutes. 100 µL of starch solution (1% starch in PBS) was added to each tube and incubated at room temperature for 3 minutes. The reaction was then stopped by adding 1000 µL of freshly produced DNS reagent to each tube and heating them on a water bath at 95°C for 5 minutes. A blank containing phosphate buffer (pH 6.9) instead of the extract or acarbose was prepared. The reaction mixture was diluted up to 2ml using Phosphate buffer. The absorbance was measured at 540 nm using UV visible spectrophotometer. The experiment is repeated thrice.

The alpha amylase inhibitory activity was calculated by using the formula [11].

% Inhibition = Abs of control – Absorbance of test / Absorbance of control ×100

Concentrations of fractions resulting in 50% inhibition of enzyme activity (IC50) were determined graphically.

5.4.2. Alpha glucosidase inhibitory assay

Sodium Phosphate buffer was used to create 1 mg/ml of enzyme, test samples, and the standard medication (Acarbose) (0.2 M pH 4.5). Test samples at 200 and 400 µg/mL concentrations were introduced to 10 µL Alpha glucosidase enzyme in 1.5 mL microcentrifuge tubes and incubated at room temperature for 5 minutes. 100 µL of 37Mm sucrose solution was added to each tube and incubated for 30 minutes at room temperature. The tubes were then heated for 5 minutes at 100 °C to cease the process. The reaction mixture was diluted to 2 ml with phosphate buffer, then 1 ml of the reaction mixture was transferred to another test tube and 1 ml if glucose oxidase peroxidase reagent was added as directed and incubated for 30 minutes. A blank containing phosphate buffer (pH 6.9) instead of the extract or acarbose was prepared. The absorbance was measured at 505 nm using UV visible spectrophotometer. The experiment is repeated thrice [12].

The alpha glucosidase inhibitory activity was calculated by using the formula:

% Inhibition = Abs of control – Absorbance of test / Absorbance of control ×100

Concentrations of fractions resulting in 50% inhibition of enzyme activity (IC50) were determined graphically.

5.4.3. Glucose Uptake Assay by Yeast Cells

The assay has been performed according to the established method of Cirillo with some modifications [13]. Commercial baker's yeast was dissolved in distilled water to make a 1% yeast suspension. The suspension was incubated at room temperature (25°C) overnight. The next day, a yeast cell suspension was centrifuged at 4200 rpm for 5 minutes. By repeating the procedure with the addition of distilled water to the pallet, a clear supernatant was obtained. A 10%v/v yeast cell suspension was made by combining exactly 10 parts clear supernatant fluid and 90 parts distilled water. Different test samples and standard drugs with concentrations ranging from 50 µg to 1600 µg were prepared in distilled water. 1 ml of 5 mM glucose was combined with 1 ml of test samples or standard drug. At 37°C, the mixture was incubated for 10 minutes. The reaction was started by adding 100 µL of yeast suspension to the sample and glucose mixture, then vertexing and incubating for 60 minutes at 37°C.After incubation, the tubes were centrifuged for 5 minutes at 3800 rpm and the absorbance has been recorded by using a UV spectrophotometer at 520 nm. The percent increase in glucose uptake was calculated by the formula:

**% increase in glucose uptake=((Abs.of control - Abs.of sample))/(Abs.of control)×100**

where control solution contains all the reagents except the test sample. Metformin was used as standard drug.

**5.5. *In-Vivo* Antidiabetic Activity**

5.5.1. Experimental Animals

A group of albino rats of either sex weighing about 160–200 g was procured from Vaarunya Biolabs Private Limited (CPCSEA Registration No. 2076/ P O/RcBi Bt/S/19/C PCSEA), Bengaluru, Karnataka, India. The animals were given free access to distilled water ad libitum throughout the experiment and fed with a standard diet. The animals were acclimated to regular laboratory temperatures (25°C) and kept on a 12:12 hour light: dark cycle. The animals were kept under standard conditions in a central animal house facility of our institution, certified by the Committee for the Control and Supervision of Animal Experiments

(CPCSEA). The study protocol was approved by Institutional Animal Ethics Committee (IAEC) of Sri Adichunchanagiri College of Pharmacy, BG Nagara (IAEC Approval No. SACCP-IAEC/2022-01/58).

5.5.2. Induction of Diabetes

To induce diabetes, the rats were given an intraperitoneal injection of freshly produced STZ (45 mg/kg bw) in 0.02 M citrate buffer (Yarow Chem Products, Mumbai, Maharashtra, India). To avoid the deadly hypoglycemia produced by STZ, rats were later given a 5% glucose solution. Diabetes was confirmed a week after STZ injection in rats with fasting plasma glucose levels of more than 250 mg/dL in this study [14].

5.5.3. Experimental Design

There were five rat groups, each with six rats. The test samples were suspended in 2.5% acacia and administered to all the rats once daily for 21 days.

Group I: Normal control (Acacia 2.5%);
Group II: Diabetic control (STZ, 45 mg/kg b.w. i.p.)
Group III: Diabetic rats treated with Centella asiatica leaf extract (200 mg/kg b.w. p.o)
Group IV: Diabetic rats treated with Zingiber officinale rhizome extract (200 mg/kg b.w. p.o)
Group III: Diabetic rats treated with Metformin (90mg/kg b.w. p.o)

5.5.4. Blood Glucose Level

The blood glucose level was tested using the "Rupturing tail vein technique" on the first, seventh, fourteenth, and twenty-first days of treatment using a one-touch glucometer (LifeScan, Inc., Malvern, PA, USA).

5.5.5. Biochemical and Histopathological Analysis

On the 21st day of the trial, blood was obtained via cardiac puncture and centrifuged for 15 minutes at 3000 rpm to assess lipid profiles such as triglycerides (TG), total cholesterol (TC), low-density lipoprotein (LDL), high-density lipoprotein (HDL), and very low-density lipoprotein (VLDL). For histological examinations, the liver and pancreas were isolated and stored in a 10% formalin solution.

5.5.6. Statistical Analysis

Data were presented as mean SEM, and statistical differences between groups were established using analysis of variance (ANOVA) and Dunnett's test. Statistical significance was defined as p-values less than 0.05.

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