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# Antidiabetic Potential of Tea Polysaccharides from Indian Cultivars Using *in vitro* and *in vivo* Evaluation

Running title: Antidiabetic Potential of Indian Tea Polysaccharides

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## SUMMARY

*Research background*. Natural polysaccharides have gained increasing attention because of their potential role in the management of diabetes mellitus. Tea is a widely consumed beverage containing structurally diverse polysaccharides that contribute to its associated health benefits. However, polysaccharides from Indian tea cultivars remain insufficiently explored, particularly regarding their structural characteristics and anti-diabetic potential.

*Experimental approach*. Fresh leaves from six Indian tea cultivars (TV-17, TV-22, Teen Ali, TRF-1, UPASI-9, and UPASI-28) were screened for polysaccharide yield using hot-water extraction followed by ethanol precipitation. Based on their higher polysaccharide content, Teen Ali and UPASI-28 were

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selected for further structural characterization by Fourier-transform infrared spectroscopy (FTIR) and scanning electron microscopy. Their antidiabetic potential was evaluated through α-amylase and α-glucosidase inhibition assays and further validated in an alloxan-induced diabetic mouse model.

*Results and conclusions*. The isolated polysaccharides exhibited characteristic structural features of carbohydrate-rich biomolecules and possessed distinct morphological properties. Both samples demonstrated significant inhibitory activity against major carbohydrate-hydrolysing enzymes, indicating their potential to regulate glucose metabolism. In vivo studies further confirmed their anti-diabetic efficacy through improved glycaemic control, recovery from diabetes-associated body mass loss, and recovery of tissue architecture in treated groups. The observed biological activity may be associated with structural attributes such as hydroxyl groups and uronic acid content, which could enhance interactions with target enzymes.

*Novelty and scientific contribution.* This study provides a comparative investigation of polysaccharides derived from Indian tea cultivars by integrating structural characterization with both in vitro and in vivo anti-diabetic evaluations. The findings contribute to the understanding of tea polysaccharides as potential natural therapeutic agents for diabetes management and highlight the biomedical relevance of underexplored Indian tea varieties.

**Keywords:** tea polysaccharides; Indian tea cultivars; antidiabetic activity; enzyme inhibition; functional food; glycemic control

## INTRODUCTION

Diabetes mellitus remains one of the most pressing global health challenges due to its rising prevalence and its association with complications such as cardiovascular disease, nephropathy, and retinopathy [*1*]. Although several pharmacological therapies are available, many are limited by side effects, high cost, or reduced long-term efficacy [*2*], prompting an increasing interest in natural, safer alternatives for glycemic management. Plant-derived polysaccharides have emerged as multifunctional bioactive compounds with immunomodulatory, antioxidant, and antidiabetic properties [*3*]. Owing to their diverse structural features—such as uronic acids, protein-bound fractions, and varied branching patterns—these macromolecules can modulate glucose metabolism through multiple mechanisms, including inhibition of carbohydrate-digesting enzymes, enhancement of insulin sensitivity, protection of

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pancreatic β-cells, and regulation of gut microbiota [*4*]. Their low toxicity and widespread occurrence make them promising candidates for functional foods and nutraceutical development.

Tea (*Camellia sinensis* (L.) Kuntze) is consumed worldwide, and although its health benefits are commonly attributed to polyphenols, recent studies highlight tea polysaccharides (TPS) as key contributors to its therapeutic potential. TPS show considerable structural variation depending on tea type, cultivar, and processing method, leading to differences in monosaccharide composition, molecular mass, and acid substitution that correlate with biological activity [*5*]. For example, TPS from Puerh tea have demonstrated hypoglycemic effects in alloxan-induced diabetic mice by enhancing antioxidant enzymes (SOD, GSH-Px) and reducing lipid peroxidation (MDA) [*6*]. Similarly, TPS have been shown to exert hypoglycemic and hypolipidemic effects in type 2 diabetic rat models through modulation of gut microbiota and metabolic pathways [*7*]. Dietary bioactive compounds such as polysaccharides have been shown to improve glycemic control in diabetic conditions [*8*]. Nevertheless, there have been few studies that examined the structural as well as biochemical characteristics of tea polysaccharides isolated from Indian varieties of tea [*9*]. Extraction conditions also influence TPS efficacy, with green tea leaf and flower polysaccharides differing in monosaccharide composition and in vitro α-glucosidase/α-amylase inhibition depending on the extraction method [*3*].

Despite these advances, TPS from Indian tea cultivars—particularly Assam and South Indian varieties—remain underexplored. These regions possess unique genetic backgrounds and agro-climatic conditions that influence tea leaf chemistry, yet few comparative studies investigate TPS from these cultivars. Previous studies have demonstrated that selenium-enriched tea polysaccharides can improve insulin resistance and reduce oxidative stress in experimental models [*10*]. However, the contribution of selenium and polysaccharide fractions to these biological effects remains difficult to distinguish, highlighting the need to investigate non-supplemented tea polysaccharides from different cultivars. Moreover, most existing reports lack an integrated approach that combines in vitro enzyme inhibition, in vivo antidiabetic evaluation, and histopathological validation.

To address these gaps, the present study investigates polysaccharides from two Indian tea cultivars, Teen Ali (Assam) and UPASI-28 (South India), evaluating their antidiabetic potential using α-amylase and α-glucosidase inhibition assays, alloxan-induced diabetic mouse models, and histological analyses to establish their efficacy and therapeutic relevance. The need for knowledge about the functional capabilities of regional tea polysaccharides is important for using the natural approach to

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control blood glucose levels. Thus, the current research is targeted towards evaluating the ability of tea polysaccharides isolated from Indian varieties through in vitro enzyme inhibition studies and in vivo animal experiments. The choice of employing enzyme inhibition studies alongside in vivo experiments was made due to the fact that such an approach would allow for a comprehensive assessment of not only the biochemical properties but also physiological significance of the obtained polysaccharides. In the present study, six Indian tea cultivars were initially screened for polysaccharide yield and preliminary antidiabetic potential. Based on the screening results, the two cultivars exhibiting the highest polysaccharide content (Teen Ali and UPASI-28) were selected for detailed physicochemical characterization and biological evaluation. Thus, the study was designed as a two-stage investigation consisting of cultivar screening followed by a comprehensive assessment of the most promising polysaccharide-rich cultivars.

## MATERIALS AND METHODS

### *Tea samples*

Fresh tea leaves from six cultivars were collected from experimental tea plantations located in Assam, India (TV-17, TV-22 and Teen Ali) and Tamil Nadu, India (TRF-1, UPASI-9 and and UPASI-28). The samples were collected during the active growing season and transported to the laboratory under ambient conditions for further processing. Collected leaves were washed thoroughly with distilled water, shade-dried at room temperature (25–28 °C), and ground into fine powder using a sterile mortar and pestle. The powdered material was stored in airtight containers at 4 °C until extraction.

### *Polysaccharide extraction*

Polysaccharides were extracted following a modified hot-water extraction and ethanol precipitation protocol. Dried powdered tea leaves (10 g) were suspended in 200 mL of distilled water at 95 °C for 2 hours. The extract was filtered and concentrated, and polysaccharides were precipitated by adding 96 % ethanol (96 %, analytical grade; Merck Life Science Pvt. Ltd., Mumbai, India) at a final concentration of 86 %. The mixture was centrifuged at 7500×*g* for 10 min at 4 °C. The precipitate was washed twice with 80 % ethanol to remove mono- and oligosaccharides, followed by drying and dissolution in distilled water. The mixture was maintained at 4 °C overnight to ensure complete precipitation and subsequently centrifuged at 7500×*g* for 10 min. The recovered precipitate was washed

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twice with 80 % ethanol, dried under vacuum, and dissolved in distilled water for further analyses. No additional purification procedures were applied. The extraction protocol was adapted from previously reported methods for tea polysaccharide isolation with minor modifications.

*Polysaccharide quantification (phenol–sulfuric acid method)*

Polysaccharide concentration was quantified using the phenol (analytical grade; Merck Life Science Pvt. Ltd., Mumbai, India)–sulfuric acid (95–98 %; Merck Life Science Pvt. Ltd., Mumbai, India) colorimetric assay with soluble starch (HiMedia Laboratories Pvt. Ltd., Mumbai, India) as the calibration standard, following Stewart [*11*] with modifications. A starch standard curve was prepared using 0, 20, 40, 60, 80, and 100 µg/mL starch solutions. To each well of a 96-well plate, 50 µL of either sample or standard was added, followed by 30 µL of 5 % phenol and 150 µL of concentrated sulfuric acid. Plates were incubated at 90 °C for 5 minutes and cooled to room temperature. Absorbance was measured at 492 nm in a Multiskan GO microplate reader (Thermo Fisher Scientific, Vantaa, Finland).

A linear regression equation was obtained using the following equation:

$$A=0.0063\cdot\gamma+0.0005 \qquad /1/$$

where $A$ represents the absorbance and $\gamma$ represents starch concentration in µg/mL. Sample absorbances were converted into starch-equivalent concentrations, and final polysaccharide yield was expressed as mg starch equivalents per gram of dry leaf mass (mg SE/g DM) using the following equation:

$$w(\text{polysaccharide})=(\gamma\cdot V)/(1000\cdot m) \qquad /2/$$

where $\gamma$ represents sample concentration (µg/mL), $V$ is the final volume of the sample (mL), and $m$ is the dry mass of the sample (g).

Where applicable, samples were diluted to fit within the linear range of the standard curve. All measurements were performed in triplicate.

*FTIR analysis*

The structural characteristics of the extracted polysaccharides were analysed using Fourier-transform infrared spectroscopy (FTIR; IRTracer-100, Shimadzu, Kyoto, Japan). Dried polysaccharide samples were mixed with spectroscopic-grade potassium bromide (KBr) (Merck Life Science Pvt. Ltd.,

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Mumbai, India), compressed into pellets, and scanned over a wavelength range of 4000–400 $cm^{-1}$ at a resolution of 4 $cm^{-1}$. The obtained spectra were used to identify characteristic functional groups associated with tea polysaccharides.

*Scanning electron microscopy*

The surface morphology of tea polysaccharides was examined using scanning electron microscopy. Dried polysaccharide samples were mounted on aluminum stubs using double-sided conductive carbon tape and sputter-coated with a thin layer of gold to improve conductivity. The coated samples were observed using a scanning electron microscope (JSM-IT200, JEOL Ltd., Tokyo, Japan) operated at an accelerating voltage of 10 kV. Micrographs were recorded at different magnifications (250, 500, 1000 and 2500×) to evaluate particle morphology, surface characteristics, aggregation patterns, and porosity of the polysaccharide samples.

*In vitro antidiabetic activity*

α-amylase inhibition assay

A 1 % (*m*/*V*) starch solution was prepared by dissolving 1 g of soluble starch in 100 mL of 20 mM phosphate buffer (pH=6.9) containing 6.7 mM NaCl. The α-amylase (HiMedia Laboratories Pvt. Ltd., Mumbai, India) enzyme solution (1 U/mL) was prepared in the same buffer. Polysaccharides were dissolved in a minimal amount of dimethyl sulfoxide (DMSO; HiMedia Laboratories Pvt. Ltd., Mumbai, India) (final solvent concentration<1 %) and diluted with phosphate buffer to obtain final test concentrations of 10, 20, 40, 60, 80, and 100 µg/mL. In a test tube, 200 µL of enzyme solution was mixed with 200 µL of polysaccharide solution and preincubated at 37 °C for 10 min. Then, 200 µL of starch solution was added to initiate the reaction and the mixture was further incubated at 37 °C for 10 min. The reaction was terminated by adding 200 µL of 3,5-dinitrosalicylic acid (DNS; SRL Chemicals, Mumbai, India) reagent (prepared by dissolving 1 g of 3,5-dinitrosalicylic acid and 30 g of sodium potassium tartrate (SRL Chemicals, Mumbai, India) in 20 mL of 2 N sodium hydroxide (NaOH; Merck Life Science Pvt. Ltd., Mumbai, India) and making up the volume to 100 mL). The tubes were then placed in a boiling water bath for 5 min, cooled to room temperature, and diluted with 2 mL of distilled water. Absorbance was measured at 540 nm using a UV–visible spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan). Acarbose (Sigma-Aldrich, St. Louis, MO, USA) (10–100 µg/mL) served as the positive control. The blank was prepared by

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replacing the enzyme solution with 200 μL of distilled water, while the control contained enzyme and substrate without inhibitor. All assays were performed in triplicate ($N$=3) and the inhibition percentage was calculated using the following equation:

$$I=[(A_c-A_s)/A_c]\cdot 100 \qquad /3/$$

where $I$ is the inhibition percentage, $A_c$ is the absorbance of the control and $A_s$ is the absorbance of the sample. Values were calculated as mean±average standard error of the mean of three repetitions [*12,13*].

α-glucosidase inhibition assay

Inhibition of α-glucosidase (Sigma-Aldrich, St. Louis, MO, USA) activity was determined using yeast α-glucosidase and p-nitrophenyl-α-D-glucopyranoside (Sigma-Aldrich, St. Louis, MO, USA) as described by Kim *et al.* [*14*]. Acarbose, polysaccharides from tea plants (10, 20, 40, 60, 80 and 100 μg/mL) were added to 50 μL of α-glucosidase (1 U/mL) prepared in 0.1 M phosphate buffer (pH=6.9), and 250 μL of 0.1 M phosphate buffer to get 0.5 to 5.0 mg/mL final concentration. The mixture was preincubated at 37 °C for 20 min. After preincubation, 10 μL of 10 mM p-nitrophenyl-α-D-glucopyranoside prepared in 0.1 M phosphate buffer (pH=6.9) was added, and incubated at 37 °C for 30 min. The reactions were stopped by adding 650 μL of 1 M sodium carbonate, and the absorbance was measured in a spectrophotometer (Shimadzu UV-1800, Kyoto, Japan) at 405 nm. The α-glucosidase inhibitor (acarbose) was used as a standard. The percentage of inhibition of α-glucosidase was determined by Eq. 3. Values are expressed as mean±standard error of the mean ($N$=3) [*12,13*]. The concentration of each test sample required to inhibit 50 % of enzyme activity ($IC_{50}$) was calculated by plotting percentage inhibition against the logarithm of inhibitor concentration and fitting the data to a sigmoidal dose–response curve using nonlinear regression (GraphPad Prism 9.0) [*15*]. All experiments were performed in triplicate, and results are presented as mean value±standard error of the mean.

*In vivo studies*

Healthy male BALB/c mice (average body mass (28±2) g) were procured from the institutional animal facility. Only male mice were used in this study to minimize biological variability. Sex-based differences were not evaluated and represent a limitation of the study. The animals were housed under standard laboratory conditions ((22±2) °C, 12 h light/12 h dark cycle, relative humidity 50–60 %) with free

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access to standard pellet diet and water ad libitum. All experimental protocols were approved by the Institutional Animal Ethics Committee Arulmigu Kalasalingam College of Pharmacy, Krishnankoil (IAEC) and conducted in accordance with CPCSEA/NIH guidelines for the care and use of laboratory animals [*16*].

Diabetes was induced by a single intraperitoneal injection of alloxan monohydrate (Sigma-Aldrich, St. Louis, MO, USA) (150 mg/kg body mass) freshly prepared in sterile normal saline. Mice were fasted overnight (12 h) before injection with free access to water. After 72 h, blood glucose levels were measured using tail vein blood and a glucometer. Mice with fasting blood glucose levels ≥200 mg/dL were considered diabetic and included in the study. Fasting blood glucose levels were measured on day 0 (baseline, post-induction), day 7, day 14, and day 21 using a handheld glucometer with blood collected from the tail vein after overnight fasting. Body mass was also recorded on the same days to monitor changes during treatment.

At the end of the 21-day treatment period, animals were fasted overnight and euthanized under IAEC-approved ethical procedures using an overdose of anaesthesia. The pancreas, liver, and kidney were immediately excised, washed with ice-cold normal saline, fixed in 10 % neutral buffered formalin for 24–48 h, processed by routine paraffin embedding, sectioned at approximately 5 μm thickness, stained with hematoxylin and eosin (H&E), and examined under a light microscope for histopathological alterations.

Experimental design

Mice were randomly divided into nine groups (*N*=6 per group) as follows: (*i*) group I (control): normal mice receiving vehicle only, (*ii*) group II (diabetic control): alloxan-induced mice receiving vehicle only, (*iii*) group III (standard): diabetic mice treated with metformin (150 mg/kg body mass/day, orally), (*iv*) group IV (low dose): diabetic mice treated with polysaccharides from Teen Ali (10 mg/kg body mass/day, orally), (*v*) group V (medium dose): diabetic mice treated with polysaccharides from Teen Ali (50 mg/kg body mass/day, orally), (*vi*) group VI (high dose): diabetic mice treated with polysaccharides from Teen Ali (100 mg/kg body mass/day, orally), (*vii*) group VII (low dose): diabetic mice treated with polysaccharides from UPASI-28 (10 mg/kg body mass/day, orally), (*viii*) group VIII (medium dose): diabetic mice treated with polysaccharides from UPASI-28 (50 mg/kg body mass/day, orally), and (*ix*) group IX (high dose): diabetic mice treated with polysaccharides from UPASI-28 (100 mg/kg body mass/day, orally). Six Indian tea cultivars were initially screened for polysaccharide yield using the

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phenol–sulfuric acid method and for their in vitro antidiabetic potential through α-amylase and α-glucosidase inhibition assays. Among the six cultivars, Teen Ali and UPASI-28 exhibited the highest polysaccharide yields (55 and 45 mg SE/g dry mass, respectively) together with the strongest inhibitory activities against both enzymes. Based on these preliminary screening results, these two cultivars were selected for detailed physicochemical characterization and comprehensive in vivo antidiabetic evaluation, while the remaining cultivars served as the initial screening group

Dose selection was based on previous investigations demonstrating the safety and biological efficacy of food-derived polysaccharides within this dosage range in experimental diabetic models [*5,10,16*]. All treatments were administered once daily for 21 consecutive days orally. Doses were calculated individually based on the average body mass of 28 g.

*Statistical analysis*

Data are presented as mean value±standard error of the mean. Prior to statistical analysis, data normality was assessed using the Shapiro–Wilk test. Since all datasets followed a normal distribution ($p>0.05$), statistical comparisons among groups were performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. Differences were considered statistically significant at $p<0.05$. Statistical analyses were performed using GraphPad Prism v. 9.0 [*15*].

## RESULTS AND DISCUSSION

*Extraction and quantification*

Quantification of total polysaccharide amounts from all tea clones revealed differences in carbohydrate content. Fig. 1a illustrates the standard curve prepared after using the phenol-sulfuric acid assay, with starch standards ranging from 0 to 100 µg/mL. The standard curve was linear

$$A=0.0063C+0.0005 \qquad /4/$$

where A represents absorbance measured at 492 nm and C represents starch concentration (µg/mL).

Fig. 1b shows the amounts of extracted polysaccharide from six different tea cultivars, expressed in mg starch equivalents (SE) per g dry mass (DM). For the different tea cultivars, mg SE was calculated using their absorbances, which corresponded to 38, 30, 55, 22, 35 and 45 mg SE per g DM for TV-17, TV-22, Teen Ali, TRF-1, UPASI-9 and UPASI-28, respectively. These results indicate that Teen Ali and

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UPASI-28 have higher amounts of polysaccharide compared to the other cultivars, which further justifies their use to explore antidiabetic potential.

The variability in polysaccharide production among the different varieties suggests the effect of genotype and the environment on carbohydrate content, as was earlier suggested by studies on the influence of the environment and variety on the composition of polysaccharides in tea plants [*7,16*].

The variability in polysaccharide yield observed among the tea cultivars is consistent with previous reports indicating that tea genotype, geographical origin, environmental conditions, and processing methods significantly influence polysaccharide accumulation. Guo *et al.* [*17*] reported considerable variation in extraction yields among twelve Chinese tea varieties, with polysaccharide yields ranging from 1.81 to 6.38 % depending on tea category and compositional characteristics. Similarly, Hu *et al.* [*8*] emphasized that differences in cultivar background and extraction conditions strongly affect the structural composition and biological functionality of tea polysaccharides. The comparatively higher polysaccharide content observed in Teen Ali and UPASI-28 may therefore reflect cultivar-specific metabolic differences associated with carbohydrate biosynthesis and storage.

*FTIR spectra of tea polysaccharides*

FTIR analysis for the various tea cultivars revealed similar polysaccharide characteristics for all the samples; for instance, the broader bond for the O-H stretch was found around 3200-3400 $cm^{-1}$, while C-H stretching for the samples was around 2920-2940 $cm^{-1}$. Teen Ali and UPASI-28 also displayed much weaker bands for the amide bands around 1630-1650 $cm^{-1}$ for the samples, indicating much lesser levels of contamination with proteins and higher purity levels of the polysaccharide samples (Fig. 2). Also, the band around 1730-1750 $cm^{-1}$ for uronic acids exhibits higher levels for the mentioned cultivars: Teen Ali and UPASI-28, while the fingerprint region around 1000-1200 $cm^{-1}$ for the glycosidic linkages presents itself very distinctly for the samples. Also, the structural integrity for the samples is prominently observed for Teen Ali and UPASI-28, which may contribute to the enhanced bioactivity observed in these samples which is associated with higher bioactivity for antidiabetic effects while serving as polysaccharides.

Moreover, the presence of uronic acids increases the strength of electrostatic interaction and enzyme binding efficiency, thus making them good inhibitors. According to recent findings, polysaccharides having uronic acids and hydroxyl groups are more efficient enzyme inhibitors than others

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owing to better molecular interactions and greater flexibility of their conformation [*16,17*]. These structural features collectively support the enhanced enzyme inhibitory activity observed in TPS samples. Similar structural characteristics have been reported for tea polysaccharides isolated from Chinese green tea and Pu-erh tea varieties, where strong hydroxyl and glycosidic linkage signals were associated with enhanced biological activities. Guo *et al.* [*17]* reported that tea polysaccharides containing abundant uronic acid residues exhibited improved antioxidant and enzyme inhibitory properties. The stronger uronic acid-associated absorption bands observed in Teen Ali and UPASI-28 may therefore contribute to their superior antidiabetic activity through enhanced interaction with digestive enzymes.

*Morphological characterization of tea polysaccharides*

Scanning electron microscopic studies showed morphological differences between polysaccharides isolated from various tea cultivars (Fig. S1). Scanning electron microscopic images showed irregular, agglomerated, and porous structures with significant differences in their surface topography and structural arrangement. The polysaccharides from TRF-1 and UPASI-9 had tight and irregular flakes with a high degree of aggregation, implying strong intramolecular bonding and less surface porosity. In contrast, TV-17 and TV-22 had amorphous and heterogeneous structures with smooth surfaces and poorly organized morphology, implying weaker intermolecular cohesion. Remarkably, UPASI-28 had a highly porous and honeycomb structure, providing a greater surface area and promoting enzyme interactions. Likewise, the polysaccharides from Teen Ali possessed granular and clustered structures with moderate surface roughness, which implies higher reactivity and better binding affinity towards enzymes.

The variation in the structure of the tea polysaccharide can greatly affect their biological properties, especially enzyme inhibition activities. Porous and loosely structured polysaccharides, such as UPASI-28 and Teen Ali, can increase interaction with α-amylase and α-glucosidase because of the larger surface area and greater substrate accessibility. It is also documented that porous and uneven structured polysaccharides provide better biological activity due to increased interaction with target enzymes and increased molecule diffusion. The higher antidiabetic activity of the polysaccharide in Teen Ali and UPASI-28 can thus be attributed to the improved microstructure of these polysaccharides. Similar structure–bioactivity relationships have been reported for plant polysaccharides, where porous morphology enhances enzyme accessibility and functional performance [*16*]. Morphological

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characteristics are increasingly recognized as important determinants of polysaccharide bioactivity. Previous investigations have shown that porous and irregular polysaccharide structures provide greater surface area for molecular interactions and facilitate improved accessibility to target enzymes. Zhu *et al.* [*18*] demonstrated that tea polysaccharide fractions possessing more porous architectures exhibited stronger hypoglycemic activity compared with compact structures. The highly porous honeycomb-like morphology observed in UPASI-28 and the granular surface organization of Teen Ali may therefore contribute to their enhanced interaction with α-amylase and α-glucosidase, resulting in improved inhibitory activity.

*In vitro antidiabetic activity of tea polysaccharides*

The studies on the inhibition of α-amylase clearly demonstrated a concentration-dependent response for the inhibitory activity for all the tea samples. Out of these tea samples, Teen Ali and UPASI-28 depicted potent inhibitory activity with 90.4 % and 89.1 %, respectively, at 100 µg/mL concentrations compared to 86.8 % for the standard drug acarbose (Table 1). Also, the calculated $IC_{50}$ from the non-linear regression method for Teen Ali and UPASI-28 samples were found to be 32.8 µg/mL and 33.5 µg/mL, respectively, which were significantly lower than the $IC_{50}$ for acarbose (42.3 µg/mL) and very low compared to the $IC_{50}$ for the other varieties (55-58 µg/mL). The one-way ANOVA analysis showed significant differences between all the treatment groups ($p<0.0001$). Statistical differences among treatments were determined using Tukey's post hoc test, where different superscript letters indicate statistically significant differences ($p<0.05$). The polysaccharides from Teen Ali and UPASI-28 exhibited significantly greater inhibitory capacity than those from other varieties and acarbose, a common medication used for treating diabetes. These results confirm the strong inhibitory potential of TPS against carbohydrate-digesting enzymes.

The α-amylase inhibitory activity recorded in the present study was comparable to, and in some cases greater than, values previously reported for tea-derived polysaccharides. Pelvan *et al.* [*19*] demonstrated that black tea polysaccharides exhibited moderate α-amylase inhibition that varied according to extraction conditions and compositional differences. Likewise, Guo *et al.* [*17*] reported significant α-glucosidase and antiglycation activities among tea polysaccharides from different tea categories, highlighting the influence of structural variability on biological performance. The comparatively

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lower $IC_{50}$ values observed for Teen Ali and UPASI-28 suggest stronger inhibitory efficiency, which may be associated with their higher polysaccharide content and favorable structural characteristics.

The strong α-glucosidase inhibitory activity observed in the present study supports previous findings demonstrating the antidiabetic potential of tea polysaccharides (Table 1). Guo *et al.* [*17*] reported that tea polysaccharides from Pu-erh tea exhibited pronounced α-glucosidase inhibitory activity and suggested that protein-bound acidic heteropolysaccharides possess enhanced biological effectiveness. Furthermore, recent reviews have highlighted that the hypoglycemic activity of tea polysaccharides is closely related to monosaccharide composition, molecular mass distribution, and uronic acid content. The superior inhibitory activity of Teen Ali and UPASI-28 may therefore result from cultivar-dependent structural features that improve enzyme binding and interfere with carbohydrate digestion.

$IC_{50}$ values observed in this study (28.6−33.5 μg/mL) appear relatively low compared to those of several other polysaccharides isolated from plants, which is suggestive of greater inhibitory efficiency. Prior investigations involving tea polysaccharides have shown that glycosidic linkage composition, molecular structure, and uronic acid content have an important influence on biological activity. Consequently, the relatively high efficiency of Teen Ali and UPASI-28 can be attributed to their relatively lower protein contamination levels, as confirmed by FTIR analysis. Similar investigations have also found that the biological activities of tea polysaccharides increase with structural purity [*18*].

The higher inhibitory effect of TPS on both α-amylase and α-glucosidase could be linked to the unique physicochemical properties and structure of the polysaccharides. The presence of uronic acid units in TPS increases the interactions between the enzymes and polysaccharides, leading to more effective enzyme inhibition. Recent evidence has shown that polysaccharides with high content of hydroxyl and uronic acid groups exert more potent enzyme inhibition due to enhanced molecular interaction and conformational dynamics [*16,17*]. Moreover, the macromolecular nature of polysaccharides is another factor contributing to the enzyme inhibition effect, which is manifested by increased viscosity. The ability of macromolecules to form a physical barrier around starch molecules restricts the access of enzymes, resulting in slower carbohydrate hydrolysis. This process is considered critical for managing postprandial blood sugar levels since the physical barrier delays the release and subsequent absorption of carbohydrates. Likewise, food polysaccharides with increased viscosity were found to regulate glucose metabolism by inhibiting digestive enzymes [*19*].

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*In vivo antidiabetic evaluation in alloxan-induced diabetic mice*

Changes in body mass of diabetic mice

The in vivo experimental model, the body mass of diabetic control mice decreased gradually from 28.7 g on day 0 to 22.0 g on day 21. However, diabetic mice treated with tea polysaccharides showed gradual recovery in body mass., it was noted that the 100 mg/kg dosage of Teen Ali increased the body mass of the diabetic mice to 30.5 g on day 21, while the 100 mg/kg dosage of UPASI-28 produced a corresponding body mass increase of 32.6 g on day 21, which is highly comparable to that of the metformin-treated groups (33.4 g) (Table 2). From this experimental result, these findings indicate that polysaccharide supplementation is effective in preventing muscle wasting syndrome. Improved insulin sensitivity results in increased protein synthesis and subsequent prevention of muscle wasting syndrome. Similar results were noted by El Adaouia Taleb *et al.* [*20*]. One-way ANOVA along with Tukey's test showed significant differences between experimental groups ($p<0.0001$). The superscript letters show that treatment with high doses of Teen Ali and UPASI-28 has significantly increased body mass of animals when compared to control diabetic group ($p<0.05$).

Blood glucose levels of diabetic mice

The blood glucose levels continued to increase in the diabetic control mice, reaching (450±30) mg/dL on day 21. Polysaccharide treatment induced a statistically significant hypoglycemic effect ($p<0.05$) in a dose- and time-dependent manner. Treatment with Teen Ali at 100 mg/kg body mass reduced the blood glucose level to (162±8) mg/dL, while UPASI-28 at the same dose of 100 mg/kg body mass exhibited marginally higher activity, where the final blood glucose was (152±7) mg/dL (Table 3). This was almost comparable to metformin, in which the blood glucose level decreased to (132±15) mg/dL. ANOVA analysis demonstrated that statistically significant differences were observed between the groups ($p<0.0001$). The Tukey's test demonstrated that all treatment groups had significant differences when compared to the control group of diabetics ($p<0.05$). Groups of high doses of both the plant extracts had significant differences in their glucose reduction compared to low doses and medium doses.

The hypoglycemic effects observed in diabetic mice are in agreement with earlier reports describing the glucose-lowering activity of tea polysaccharides. Xu *et al.* [*5*] demonstrated that oral administration of Pu-erh tea polysaccharides significantly reduced blood glucose levels and enhanced antioxidant defence mechanisms in alloxan-induced diabetic mice. Similarly, Ren *et al.* [*10*] reported that

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selenium-containing tea polysaccharides improved insulin resistance and reduced oxidative stress in experimental diabetic models. Although differences in cultivar type and polysaccharide composition may influence efficacy, the present findings support the growing evidence that tea polysaccharides contribute to glycaemic regulation through multiple complementary mechanisms.

Histopathological analysis diabetic mice

Histopathological observations further confirmed the biochemical findings. The diabetic control mouse tissue sections showed that the pancreatic tissues of diabetic control mice were extensively damaged, evidenced by the shrinking of the islet of Langerhans and disorganized structures. On the other hand, the treated mouse tissue sections that received the polysaccharide extract exhibited well-organized structures in the pancreatic tissue sections, particularly the tissue sections that received the medium and high dosages of Teen Ali and UPASI-28 (Fig. 3). The diabetic control mouse tissue sections showed that the livers of diabetic mice were extensively damaged, characterized by steatosis, inflammation, and disorganized structures. However, the treated mice exhibited well-organized structures, particularly in the tissue sections that received the polysaccharide extract from medium and high dosages of Teen Ali and UPASI-28. The tissue sections also showed that the kidney tissues of diabetic control mice were extensively damaged, characterized by hypertrophy and congestion. On the other hand, the treated mice tissue sections exhibited well-organized structures in the polysaccharide-treated groups (Fig. 4). The livers of diabetic control mice displayed a significant amount of histopathological changes, such as steatosis, infiltration of inflammatory cells, and deranged hepatic architecture. On the other hand, tea polysaccharide intervention significantly enhanced liver architecture with decreased lipid deposition and restored cellular organization. This enhancement was more evident in the medium and high doses of Teen Ali and UPASI-28, suggesting that they have a protective role against diabetes-related liver injury (Fig. 5).

Histopathological recovery of pancreatic, hepatic, and renal tissues further supports the biological activity of tea polysaccharides beyond digestive enzyme inhibition. Previous studies have demonstrated that tea polysaccharides can protect pancreatic β-cells from oxidative damage, reduce inflammatory responses, and improve tissue architecture in diabetic conditions. Wang *et al.* [*21*] reviewed evidence indicating that tea polysaccharides modulate antioxidant defence pathways and reduce reactive oxygen species accumulation, thereby limiting diabetes-associated tissue injury. The restoration of normal tissue

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organization observed in the present study is consistent with these reported protective effects and suggests a broader therapeutic role for tea-derived polysaccharides in diabetes management.

The protective mechanism of tea polysaccharides may be attributed to their antioxidant activity, particularly through ROS scavenging mechanisms and reduction of oxidative stress, which would prevent apoptosis of pancreatic β cells and avoid subsequent damage to some organs. The antioxidant activity of natural polysaccharides has been known to function through the anti-apoptotic activity of Bcl-2 and decreased levels of pro-apoptotic factors in diabetic tissues [*22*].

In vivo findings further support the functional efficacy of TPS, as evidenced by significant reductions in fasting blood glucose levels, improvement in body mass, and restoration of tissue architecture in diabetic mice. These effects may be attributed to multiple mechanisms, including improved glucose utilization, protection of pancreatic β-cells from oxidative stress, and modulation of insulin signaling pathways. Recent studies have shown that tea polysaccharides can regulate key metabolic pathways such as PI3K/Akt and improve antioxidant defense systems, thereby contributing to glycemic control and tissue protection [*23*]. The presence of statistically significant results in both in vitro and in vivo investigations ($p<0.05$) ensures that the biological actions of the tea polysaccharides are indeed not a result of random chance but indicate their genuine pharmacological actions. The distinct statistical grouping of Teen Ali and UPASI-28 further supports cultivar-dependent variation in antidiabetic activity, likely due to differences in molecular structure and composition.

*Limitations of the study*

Although the present study demonstrates promising antidiabetic activity of tea polysaccharides, several limitations should be acknowledged. Structural characterization was limited to FTIR spectroscopy and scanning electron microscopy, and therefore important parameters such as monosaccharide composition, molecular mass distribution, glycosidic linkage patterns, and quantitative uronic acid content were not determined. Consequently, the proposed structure–activity relationships should be regarded as preliminary. In addition, no further purification procedures were performed following extraction, and the obtained polysaccharide preparations may still contain co-extracted compounds such as proteins, polyphenols, and other bioactive constituents that could contribute to the observed biological activities. Furthermore, the molecular mechanisms responsible for glucose regulation were not investigated. The biological activity was evaluated only in an alloxan-induced mouse model, and extrapolation to humans

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should therefore be approached with caution. Future studies should focus on advanced structural characterization, purification, mechanistic investigations, and clinical validation.

## CONCLUSIONS

The present study demonstrated that tea polysaccharides isolated from Indian tea cultivars possess considerable antidiabetic potential. Among the six cultivars evaluated, Teen Ali and UPASI-28 exhibited the highest polysaccharide yields and the strongest inhibitory activity against α-amylase and α-glucosidase. These findings were further supported by significant improvements in fasting blood glucose levels, recovery from diabetes-associated body mass loss, and restoration of tissue architecture in diabetic mice. The results highlight the importance of cultivar-dependent variation in determining the biological activity of tea polysaccharides. Nevertheless, further studies involving detailed structural characterization, mechanistic investigations, and clinical evaluation are required before their potential application as functional food ingredients or nutraceuticals can be fully established.

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## ETHICS APPROVAL

All experimental procedures involving animals were conducted in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New

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Delhi, India. The study protocol was reviewed and approved by the Institutional Animal Ethics Committee (IAEC) of Arulmigu Kalasalingam College of Pharmacy, Tamil Nadu, India (Approval No. AKCP/IAEC/97/20-21; Approval Date: 20.11.2021).

## CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

## AUTHORS' CONTRIBUTIONS

N. Pitcham conceived the study, performed experiments, analyzed data, and drafted the manuscript. N. Arumugam contributed to methodology, data analysis, and manuscript review. H. Natarajan contributed to study design, supervision, and project administration, and reviewed the manuscript. All authors approved the final version and are accountable for the work.

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Table 1. In vitro inhibition of α-amylase and α-glucosidase by different concentrations of tea polysaccharides

| *γ*(tea polysaccharide)/(μg/mL) | TV-17 | TV-22 | Teen Ali | TRF-1 | UPASI-9 | U-28 | Acarbose |
|---|---|---|---|---|---|---|---|
| In vitro α-amylase inhibitory activity/% | | | | | | | |
| 10 | (8.9±0.4)$^{c}$ | (9.2±0.5)$^{c}$ | (12.5±0.6)$^{a}$ | (9.6±0.5)$^{c}$ | (9.8±0.5)$^{c}$ | (11.7±0.6)$^{b}$ | (12.3±1.1)$^{a}$ |
| 20 | (20.3±0.7)$^{c}$ | (21.5±0.8)$^{c}$ | (32.8±0.9)$^{a}$ | (22.1±0.8)$^{c}$ | (23.4±0.9)$^{c}$ | (31.4±0.8)$^{b}$ | (25.4±1.1)$^{b}$ |
| 40 | (35.6±0.8)$^{c}$ | (36.9±0.9)$^{c}$ | (56.7±1.0)$^{a}$ | (38.2±0.9)$^{c}$ | (39.5±0.9)$^{c}$ | (55.2±0.9)$^{b}$ | (39.1±1.2)$^{c}$ |
| 60 | (48.7±1.0)$^{c}$ | (49.8±1.0)$^{c}$ | (73.5±1.2)$^{a}$ | (51.2±1.0)$^{c}$ | (52.6±1.0)$^{c}$ | (72.1±1.1)$^{b}$ | (52.6±1.4)$^{c}$ |
| 80 | (59.2±1.1)$^{c}$ | (60.5±1.1)$^{c}$ | (85.6±1.1)$^{a}$ | (61.9±1.1)$^{c}$ | (63.1±1.1)$^{c}$ | (84.7±1.0)$^{b}$ | (70.2±1.5)$^{b}$ |
| 100 | (67.8±1.2)$^{c}$ | (68.9±1.2)$^{c}$ | (90.4±1.2)$^{a}$ | (69.5±1.2)$^{c}$ | (70.7±1.2)$^{c}$ | (89.1±1.2)$^{a}$ | (86.8±0.8)$^{b}$ |
| In vitro α-glucosidase inhibitory activity/% | | | | | | | |
| 10 | (10.2±0.4)$^{c}$ | (10.8±0.5)$^{c}$ | (14.3±0.6)$^{a}$ | (11.5±0.5)$^{c}$ | (12.1±0.5)$^{c}$ | (13.5±0.5)$^{b}$ | (15.2±1.0)$^{a}$ |
| 20 | (23.6±0.7)$^{c}$ | (24.2±0.8)$^{c}$ | (35.7±0.9)$^{a}$ | (25.1±0.8)$^{c}$ | (26.4±0.8)$^{c}$ | (34.2±0.8)$^{b}$ | (28.7±1.2)$^{b}$ |
| 40 | (38.4±0.9)$^{c}$ | (39.6±0.9)$^{c}$ | (60.8±1.0)$^{a}$ | (40.8±0.9)$^{c}$ | (42.2±0.9)$^{c}$ | (59.4±0.9)$^{b}$ | (42.4±1.3)$^{c}$ |
| 60 | (51.6±1.0)$^{c}$ | (52.7±1.0)$^{c}$ | (76.4±1.1)$^{a}$ | (53.9±1.0)$^{c}$ | (55.4±1.0)$^{c}$ | (75.1±1.0)$^{b}$ | (55.9±1.4)$^{c}$ |
| 80 | (62.5±1.1)$^{c}$ | (63.8±1.1)$^{c}$ | (87.5±1.0)$^{a}$ | (65.1±1.1)$^{c}$ | (66.8±1.1)$^{c}$ | (86.2±1.0)$^{b}$ | (73.6±1.5)$^{b}$ |
| 100 | (70.8±1.2)$^{c}$ | (71.9±1.2)$^{c}$ | (92.7±1.2)$^{a}$ | (72.4±1.2)$^{c}$ | (73.6±1.2)$^{c}$ | (91.4±1.1)$^{a}$ | (89.1±0.9)$^{b}$ |

Values are expressed as mean value±standard error of the mean, *N*=3. Different superscript letters indicate statistically significant differences ($p<0.05$) according to one-way ANOVA followed by Tukey's post hoc test

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Table 2. Changes in body mass of control, diabetic, and polysaccharide-treated mice during 21 days of the experiment

| Sample | *t*(treatment)/day | | | |
|---|---|---|---|---|
| | 0 | 7 | 14 | 21 |
| | *m*(body)/g | | | |
| Control | (33.2±1.5)$^{a}$ | (33.8±1.4)$^{a}$ | (34.5±1.6)$^{a}$ | (35.0±1.5)$^{a}$ |
| Toxic (alloxan-induced) | (28.7±2.6)$^{b}$ | (26.0±2.4)$^{c}$ | (25.5±2.3)$^{c}$ | (22.0±2.2)$^{c}$ |
| Standard (metformin) | (32.5±1.7)$^{a}$ | (30.7±1.6)$^{b}$ | (33.0±1.5)$^{a}$ | (33.4±1.5)$^{a}$ |
| Teen Ali low dose (10 mg/kg) | (29.3±2.2)$^{b}$ | (27.8±2.1)$^{c}$ | (28.2±2.0)$^{b}$ | (28.8±2.0)$^{b}$ |
| Teen Ali medium dose (50 mg/kg) | (28.6±2.9)$^{b}$ | (26.5±2.7)$^{c}$ | (28.7±2.5)$^{b}$ | (29.9±2.4)$^{b}$ |
| Teen Ali high dose (100 mg/kg) | (27.5±2.8)$^{b}$ | (25.8±2.6)$^{c}$ | (28.2±2.4)$^{b}$ | (30.5±2.3)$^{b}$ |
| UPASI-28 low dose (10 mg/kg) | (29.0±2.4)$^{b}$ | (27.9±2.2)$^{c}$ | (29.6±2.1)$^{b}$ | (30.2±2.0)$^{b}$ |
| UPASI-28 medium dose (50 mg/kg) | (28.4±2.6)$^{b}$ | (27.0±2.3)$^{c}$ | (30.8±2.2)$^{a}$ | (31.7±2.1)$^{a}$ |
| UPASI-28 high dose (100 mg/kg) | (27.8±2.7)$^{b}$ | (26.5±2.4)$^{c}$ | (31.4±2.1)$^{a}$ | (32.6±2.0)$^{a}$ |

Values are expressed as mean value±standard error of the mean (*N*=6). Different superscript letters within the same column indicate statistically significant differences ($p<0.05$) using one-way ANOVA followed by Tukey's post hoc test

Table 3. Fasting blood glucose levels measured at 0, 7, 14 and 21 days in all experimental groups

| Sample name | *t*(treatment)/day | | | |
|---|---|---|---|---|
| | 0 | 7 | 14 | 21 |
| | (*m*(glucose)/*V*(blood))/(mg/dL) | | | |
| Control | (90±6)$^{c}$ | (92±7)$^{c}$ | (89±6)$^{c}$ | (91±5)$^{c}$ |
| Toxic (alloxan-induced) | (320±15)$^{a}$ | (365±20)$^{a}$ | (410±25)$^{a}$ | (450±30)$^{a}$ |
| Standard (metformin) | (318±12)$^{a}$ | (270±10)$^{b}$ | (200±18)$^{b}$ | (132±15)$^{b}$ |
| Teen Ali low dose (10 mg/kg) | (310±14)$^{a}$ | (282±18)$^{b}$ | (240±16)$^{b}$ | (220±19)$^{b}$ |
| Teen Ali medium dose (50 mg/kg) | (315±16)$^{a}$ | (250±15)$^{b}$ | (220±12)$^{b}$ | (190±10)$^{b}$ |
| Teen Ali high dose (100 mg/kg) | (312±13)$^{a}$ | (246±12)$^{b}$ | (208±10)$^{b}$ | (162±8)$^{b}$ |
| UPASI-28 low dose (10 mg/kg) | (311±15)$^{a}$ | (285±17)$^{b}$ | (245±14)$^{b}$ | (209±16)$^{b}$ |
| UPASI-28 medium dose (50 mg/kg) | (314±14)$^{a}$ | (248±13)$^{b}$ | (211±11)$^{b}$ | (192±10)$^{b}$ |
| UPASI-28 high dose (100 mg/kg) | (313±13)$^{a}$ | (242±12)$^{b}$ | (198±9)$^{b}$ | (152±7)$^{b}$ |

Values are expressed as mean value±standard error of the mean (*N*=6). Different superscript letters within the same column indicate statistically significant differences ($p<0.05$)

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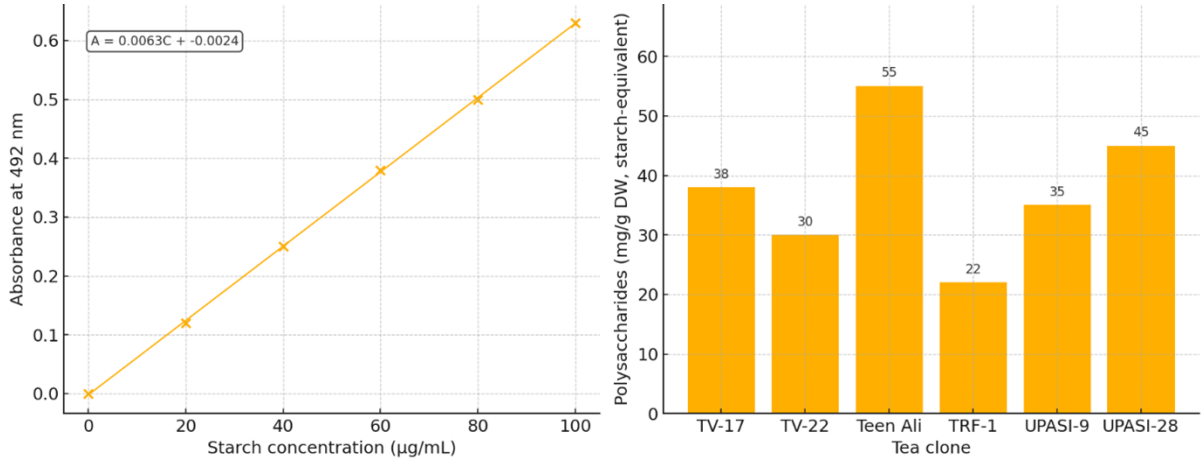


Fig. 1. (a) Standard curve of starch obtained using the phenol–sulfuric acid method. (b) Polysaccharide yield of six tea cultivars expressed as mg starch equivalents/g dry mass

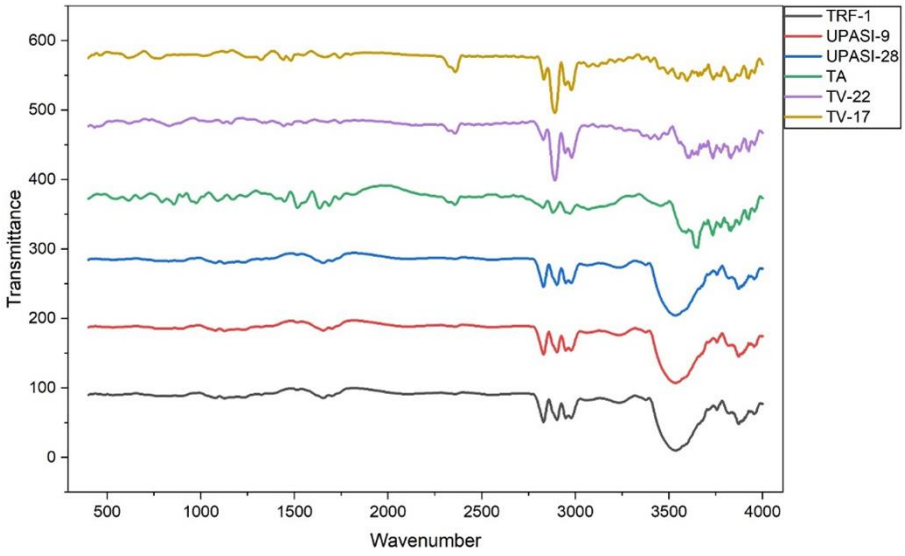


Fig. 2. FTIR spectra of polysaccharides extracted from different tea cultivars showing characteristic functional groups including hydroxyl, C–H, amide, uronic acid, and glycosidic linkages

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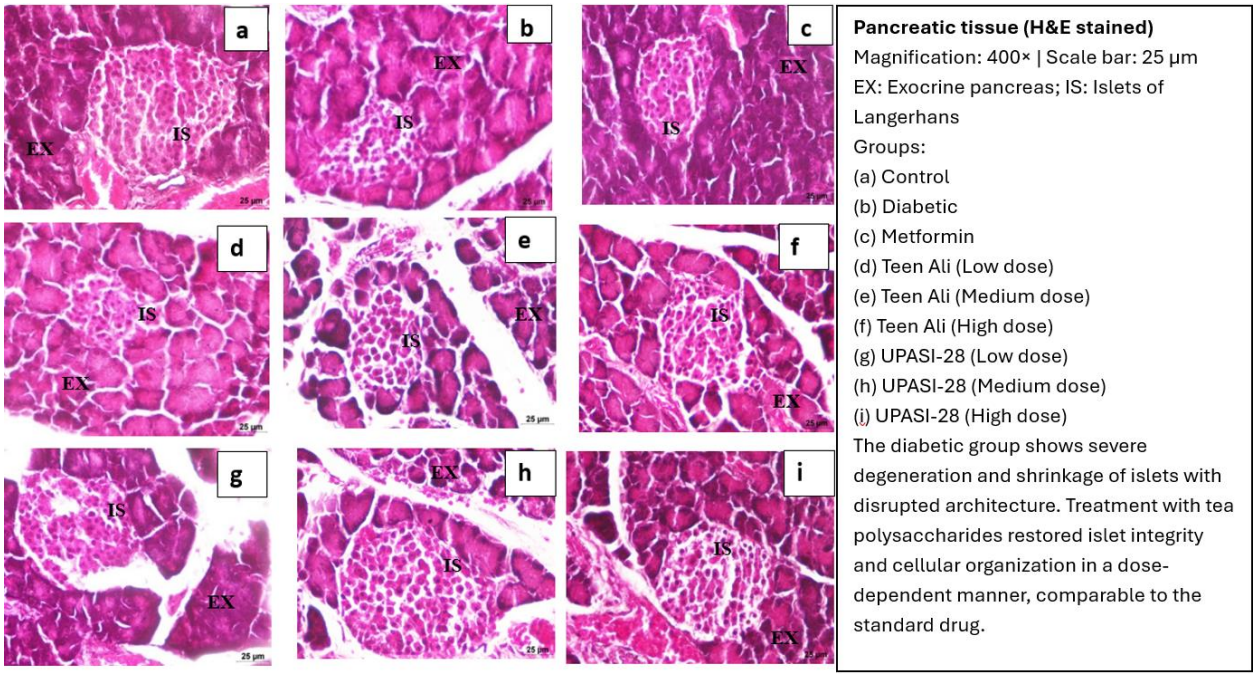


Fig. 3. Histopathological sections of pancreatic tissue in control, diabetic, and polysaccharide-treated mice showing structural alterations and restoration of islet architecture following treatment

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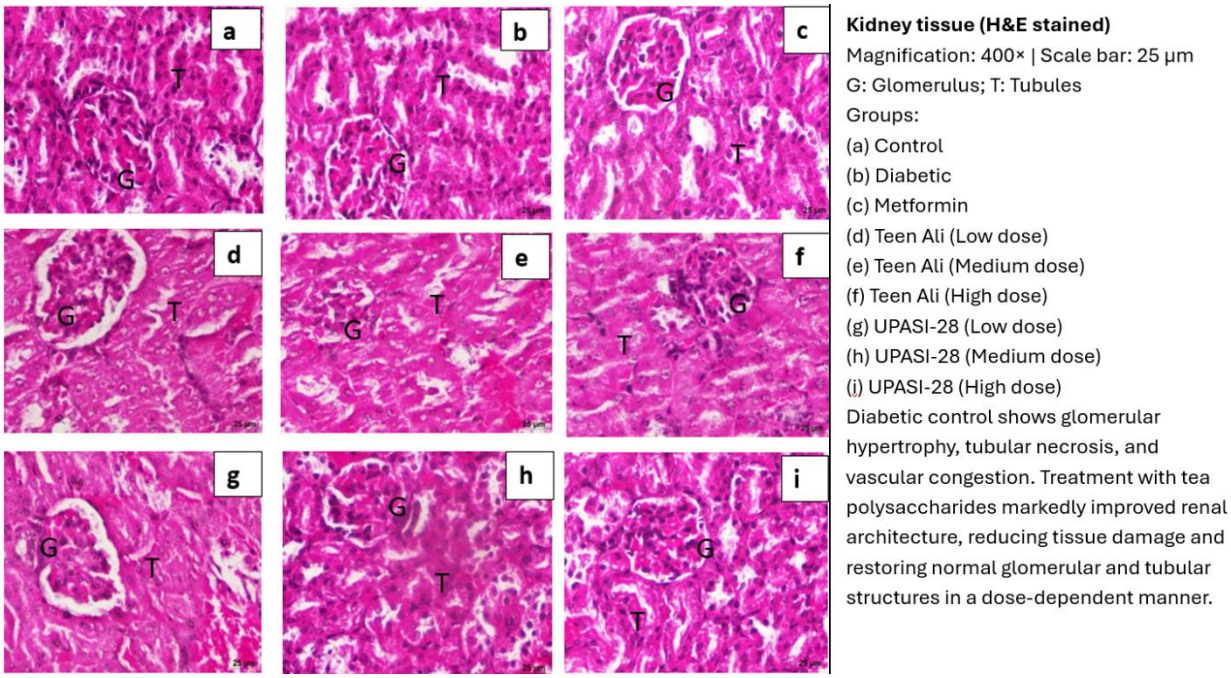


Fig. 4. Histopathological sections of kidney tissue in control, diabetic, and polysaccharide-treated mice showing tissue damage in diabetic control and improved structural integrity after treatment

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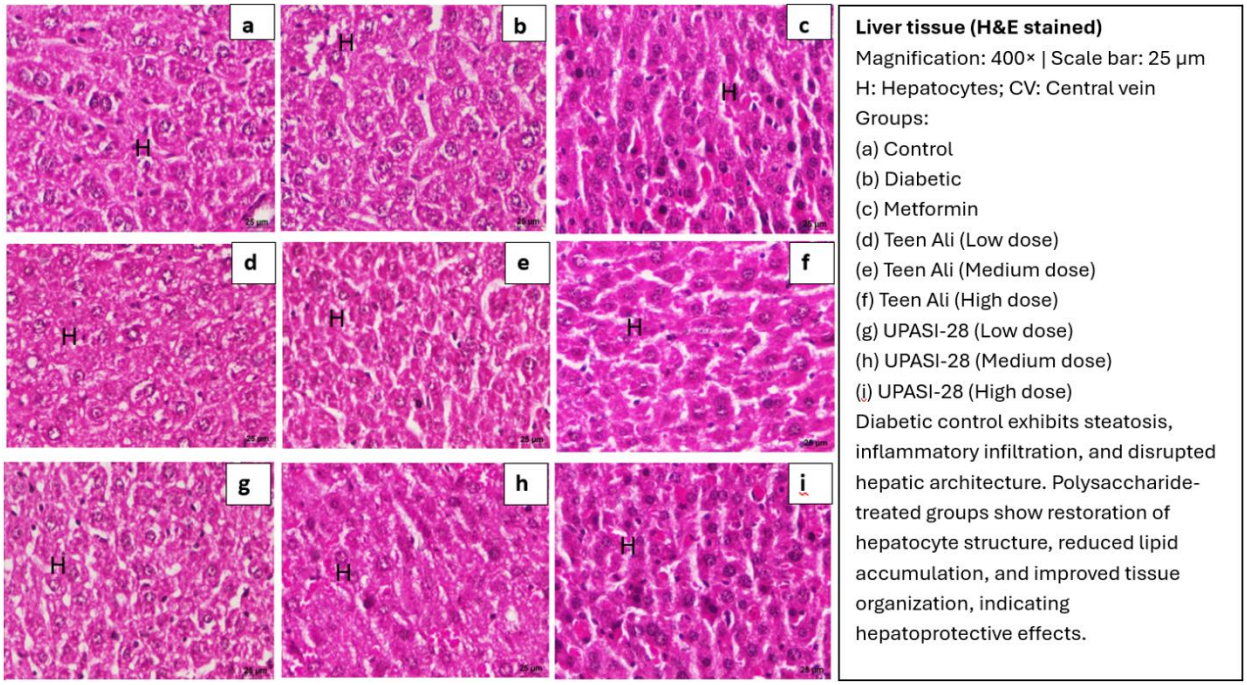


Fig. 5. Histopathological sections of liver tissue in control, diabetic, and polysaccharide-treated mice showing steatosis and inflammation in diabetic control and restored hepatic architecture in treated groups

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## SUPPLEMENTARY MATERIAL

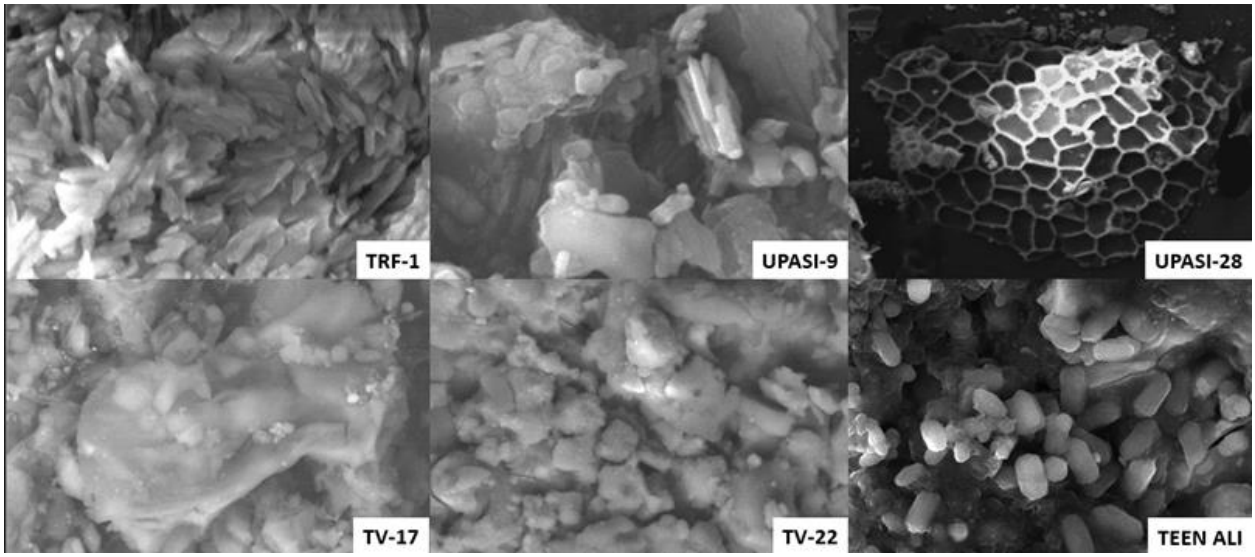


Fig. S1. Scanning electron micrographs (250, 500, 1000 and 2500×) of tea polysaccharides from different cultivars showing variations in surface morphology, including porous, aggregated and amorphous structures