مجلة دراسات وبحوث التربية النوعية

Evaluation of Anti-Diabetic and Sensory Effect of Fermented Rayeb Milk Fortified with Nano-Lemon Grass in Male Rats

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ABSTRACT

Lemongrass (Cymbopogon citratus DC, Stapf.), a member of family Poaceae, is commonly used to cure several diseases. Lemongrass aqueous extract (LGE) and LGE-loaded chitosan nanoparticles (LGE-CSNPs) exhibited potential effectiveness as antioxidant, anti-diabetic, and protective effects in male rats. Reducing power, free radical scavengers and Haemolytic activity of LGE and LGE-CSNPs were evaluated on protein levels. Total phenolics and total flavonoid contents were studied using (HPLC). Six groups of thirty-six adult male albino rats were given three different measurements of C. citratus LGE-CSNPs orally. Antioxidant activities and oxidative stress of LGE-CSNPs were evaluated on protein levels. Diabetic parameter (HBA1C) was determined. Pancreatic histopathological examinations were also studied. The sensory properties of this blind as a food supplement to fermented milk (Rayeb milk) were also evaluated. The polyphenol fingerprint of LGE revealed that chlorogenic acid (14.56 mg/ml), and quercetin (18.25 mg/ml) were the major polyphenols present in this extract. Glutathione peroxidase (GPx) of LGE-CSNPs as antioxidant activity was more active than LGE. Thiobarbituric Acid Reactive Substance (TABRAS) as oxidant concentrations and free radical scavengers have been decreased in the reference drug (Metformin), LGE, and LGE-CSNPs-treated rat groups compared to the control group (untreated). LGE-CSNPs (5 mg/kg Bwt) exhibited the lowest levels of TABRAS and (GPx). Diabetes parameters have been decreased in LGE-CSNPs (5 mg/kg Bwt) (5.13) in comparison with the reference drug (5.62). Results suggested a natural nutritional supplement that improves diabetics' condition of health. It is recommended to be added to fermented milk (Rayeb milk) as a nutritional supplement.

Keywords: Diabetes, Lemongrass, Chitosan nanoparticles, Oxidative stress, ROS, Food supplementary, Sensory evaluations

تقييم التأثير المضاد لمرض السكري والتقييم الحسي لخليط اللبن الرائب مع مستحضر عشبة

الليمون النانوية في ذكور جرذان التجارب

المستخلص:

ينتشر استخدام عشبة الليمون (Cymbopogon citratus)، عائلة (Poaceae) لعلاج مجموعة واسعة من الأمراض. لذلك، في هذه الدراسة، افترضنا أن المستخلص المائي لعشبة الليمون (LGE) والجسيمات النانوية الشيتوزان المحملة بـ (LGE-CSNPs) LGE لهما تأثيرات مضادة للأكسدة فعالة محتملة، بالإضافة إلى تأثيرات وقائية مضادة لمرض السكري في ذكور الفئران. تم تقييم قوة الاختزال، الجذور الحرة والنشاط الانحلالي لـ LGE وCSNPs وLGE-CSNPs، على مستويات البروتين. تم إجراء تحليل الفينولات الكلية ومحتويات الفلافونويد الكلية باستخدام تحليل كروماتوجرافي سائل عالي الأداء (HPLC) تم تقييم أنشطة مضادات الأكسدة والإجهاد التأكسدي لـ LGE-CSNPs على مستويات البروتين. تم قياس مستوى السكري بواسطة

. (HBAIC) كما تمت دراسة البنكرياس والفحوصات النسيجية المرضية. كما تم تقييم الخصائص الحسية نانو عشبة الليمون كمكمل غذائي للحليب المتخمر (حليب الرايب). كشف تحليل بصمة البوليفينول لـ LGE أن حمض الكلوروجينيك (٦٤,٥٦ ملغم/مل)، والكيرسيتين (١٤,٥٠ ملغم/مل)، والكيرسيتين (١٤,٥٠ ملغم/مل)، والكيرسيتين (١٤,٥٠ ملغم/مل)، والإبيكاتشين (٢,٥٩ ملغم/مل) كانت البوليفينول الرئيسية الموجودة في هذا المستخلص. تم تقدير الجلوتاثيون بيروكسيديز (GPx) الخاص بـ LGE-CSNPs كنشاط (TABRAS) مضاد للأكسدة ليكون أكثر نشاطًا من LGE، الخفض حمض الثيوباربيتوريك (TABRAS) مضاد للأكسدة ليكون أكثر نشاطًا من LGE، الموجودة في محماد للأكسدة والجذور الحرة في مجموعة الدواء المرجعي (ميتفورمين)، ومجموعات الفئران المعالجة بـ EGL و RAPC-SNPs مقارنة بالمجموعة الصابطة (غير المعالجة). أظهرت كمادة مؤكسدة والجذور الحرة في مجموعة الدواء المرجعي (ميتفورمين)، ومجموعات الفئران المعالجة بـ EGL ملغم/ كغم من وزن الجسم) أدنى مستويات ABRAS واعلي مستويات الفران المعالجة بـ EGL ملغمر من وزن الجسم) أدنى مستويات (GPX)، مقارنة بالمجموعة الصابطة (غير المعالجة). أظهرت (GPX) معارنة بالمجموعة الحران (قرب المعالجة). أظهرت المعالجة بـ EGL ملغم/ كغم من وزن الجسم) أدنى مستويات ABRAS واعلي مستويات ABRAS واعلي مستويات الفئران (GPX) مقارنة بالمجموعة الصابطة (غير المعالجة). أظهرت المعالجة بـ EGL ملغم/ كغم من وزن الجسم) أدنى مستويات ABRAS واعلي مستويات الفران (GPX) مقارنة بالمجموعة الدواء المرجعي (مرب النابل النابلي)، أطهرت مستويات الفلاري في زول الجسم) أدنى مستويات ABRAS واعلي مستويات الفران (GPX) مقارنة بمجموعة الدواء المرجعي (ميتفورمين) (٢٠٦٥)، أشارت النتائج إلى أن نانو (GPX) مقارنة بمجموعة الدواء المرجعي (ميتفورمين) (٢٠٦٥)، أشارت النتائج إلى أن نانو (GPX) معارنة بالمجموعة الدواي الحرمي (المرمي)، ومجموعات الفران (GPX) معمر / معاري مستويات الصابحة (GPX)، مألوت المعاري وروركان (GPX)، مقارن المعاري وروركا معاري)، مرز (وليا المرم)، والوليا مستويات الفلي (GPX)، منون إلى مالولي المعاري وروري)، مالولي المولي مالولي المولي مالولي المولي مالولي مالولي المولي مالولي (GPX)، منون إلى مالولي المولي مالوليا، (GPX)، مالولي المولي مالولي المولي مالولي المولي مالولي، (ارم)، مالولي مالولي المولي مالولي مالولي م

الكلمات المفتاحية: مرض السكري، عشبة الليمون، جزيئات الشيتوزان النانوية، الإجهاد التأكسدي، ROS، المكملات الغذائية، التقييمات الحسية.

Introduction

Diabetes mellitus (DM), a class of metabolic diseases, is characterised by frequently elevated blood glucose levels. One to eleven persons all over the world are suffered from DM which represents a significant global burden (Papademetriou et al., 2020). Either insufficient insulin synthesis, insufficient cell responsiveness to the insulin produced, or both are the causes of DM (Song et al., 2022). DM, the most prevalent kind of disease, is caused by low levels of insulin or an increase in insulin resistance and both of which result in hyperglycaemia (Ramraj et al., 2019). Despite the development of various traditional drugs to treat DM, plants are considered ad a feasible treatment due to their advantage side effects (Padhi et al., 2019). Medicinal plants are rich in phytochemical compounds that considered as strong therapeutic potential and minimal side effects, so they have long been used to treat a variety of illnesses and disorders including DM (Nazarian-Samani et al., 2018; Salehi et al., 2019). Herbal medicine is widely employed as an alternative therapy nowadays because of its low toxicity and inexpensive cost compared to popular medical therapy. The two main therapeutics used in nephroprotection, avoiding dialysis or kidney transplantation, are derived from herbal therapy (Huang et al., 2018). Conversely, a diet rich in high antioxidants such as fruits and vegetables reduces inflammation and inhibits the production of reactive oxygen species (ROS) and thus enhancing kidney function and postpones the beginning of chronic renal failure (Carrero et al., 2020; Zhao et al., 2021). C. citratus also known as lemongrass, is one of the plants that most commonly grown in tropical climates and exhibited several medicinal uses (Moustafa et al., 2021). C. citratus exhibited antimicrobial, antioxidant, antiseptic, anti-inflammatory, analgesic, antiamebic, antidiarrheal, antifilarial, antimalarial, antimycobacterial, anti-nociceptive, anti-protozoan, ascaricidal, and hypocholesterolemic properties (Lawal et al., 2017; Oladeji et al., 2019). Folk medicine claimed that C. citratus could treat a variety of ailments including diabetes, malaria, flu, fever, indigestion, inflammation and cough (Majewska et al., 2019; Borges et al., 2021). Medicinal and aromatic plant extracts have been discovered to contain several bioactive chemicals, among which phenolic and flavonoid compounds have been demonstrated to have antiproliferative and palliative activity. activities and other pharmacological characteristics antimicrobial (Fernández et al., 2021; Acar et al., 2023). Lemon grass has many

bioactive chemical compounds including essential oils, saponins, tannins, alkaloids, terpenoids, phenols, flavonoids, and carbohydrates (Geetha and Geetha 2014; El-Hefny et al., 2023). Several phenolic chemicals including *p*-coumaric acid, chlorogenic acid, feruloylquinic acid, and caffeoylquinic acid were detected in C. citratus leaf extract (Tavares et al., 2015). Additionally, hydroxycinnamic acid derivatives, caffeine, luteolin, apigenin, flavan derivatives of luteoliflavans, and apigeniflavans were also identified in C. citratus (Da Ressurreição et al., 2022). Several investigations had been focused on the utilization of nanoparticles encapsulating natural plant extracts which had been used as antioxidant as well as antimicrobial agents in nutraceutical applications (Armendáriz-Barragán et al., 2016). Plant extract-encapsulated polymeric nanoparticles were applied in food, pharmaceutical, cosmetics and health sectors (Armendáriz-Barragán et al., 2016; Abdou et al., 2019). The biological activity of plant extracts is increased. Various formulations based on polymeric nanoparticles and plant extracts exhibited many biological activities including cosmetology, antibacterial, anticancer, antidiabetic, antihypertensive, etc.) (Armendáriz-Barragán et al., 2016). Alginate and poly (*\varepsilon*-caprolactone) were utilized as carrier materials for white tea extract-loaded NPs that are used in nutraceutical applications (Sanna et al., 2015). The advantages plant extraction processes should improve solubility, bioavailability, enhanced stability, protection from toxicity, sustained delivery, enhanced pharmacological activity, improved tissue macrophage distribution, and protection from degradative processes (Mosaddik et al., 2018). Chitosan is a cationic linear polymer that has a high potential for encapsulating natural compounds. Chitosan is perfect for in vivo use in biomedical treatments because of its non-toxic effect (safe) as well as its antibacterial properties (Vahedikia et al., 2019). Chitosan nanoparticles (CNPs) have been widely employed to encapsulate various bioactive compounds, such as extracted essential oils (Eos) from aromatic plants (Song et al., 2021; Karimirad 2019). Therefore, this work is aimed to create current and efficient strategies for managing DM from natural, bioactive, and ecofriendly constituents. Encapsulating active compounds in a polymer matrix offers several advantages, such as regulated release and protection from the surrounding medium or processing conditions. The purpose of this research is to assess the bioactivities of lemongrass aqueous extract as well as CNPs loaded with lemongrass. Furthermore, the preventive effects against diabetes, antioxidants, in male rats were assessed. The ultimate objective is to evaluate its nutritional supplement characteristics for fermented milk (Rayeb milk).

Material and methods

Chemicals and kits

MRS broth medium (Microbiologics, USA), chitosan and Aldrich, USA) TPP (Sigma HBA1C kits triphoshphate (Hiprobiotechnology, China), oxidant, and antioxidant kits (glutathione peroxidase (GPx), , thiobarbituric acid-reactive substances (TBARS), (abcam, UK), and Folin-Ciocalteu reagent, (+)-catechin, L-ascorbic acid, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Merck SA, Germany). Fresh milk was obtained from buffaloes from the farm of faculty of agriculture, Cairo University. Lactobacillus casei HQ177095 and L. paracasei HQ177096.1 were previously isolated and identified (Khaled Elbanna Gamal, 2010; Khider et al., 2017).

Plant source and used parts

Lemongrass (*Cymbopogon Citratus* DC, Stapf.), a member of family *Poaceae*, leaves were taken from Basateen Research Center, Mansoura, Dakahlia, Egypt. The plant was deposited with voucher number of NE001 at Home Economic Department, Faculty of Specific Education, Alexandria, Alexandria University Egypt.

Lemongrass leaves extraction

Lemongrass aqueous extract (LGE) was prepared according to the modified method of **Kim et al.**, (2013). Leaves of lemongrass were dried at 40°C for 24h, then crushed into powder using a blinder. 50 g of leaf powdered were immersed in deionized water (1:20 w/v) and stirred at 45°C for 1 h. The mixture was left on magnetic stirrer for 24 h at room temperature using magnetic stirrer (Model F20500010; made in Europe). The produced mixture was filtrated through Whatman No. 1 and centrifuged at 3000×g for 15min at 4°C. The produced filtrate was freeze dried (Vacuum freeze dryer model: FDF 0350; Korea), weighed, and the extraction yield was detected and stored at 4°C till further uses.

Preparation of the chitosan-tripolyphosphate nanoparticles

Ionic gelation procedure was used to prepare chitosantripolyphosphate nanoparticles CS-TPP NPs (Fàbregas et al., ; Calvo et al., 199V). Several different concentrations of CS solutions $\cdot, 7\circ$ - 5 mg/mL were prepared in 2% solution of acetic acid under sonication for 15min at 25°C. The CS solution was then filtered (0.22µm syringe filter) to discard any un-dissolved CS. Different concentrations (0.25, 0.5, 1, 1.5, 2 and 5 mg/mL) of TPP was prepared by dissolving it in distilled water. Finally, 6 mL of TPP solution was mixed with 15 mL of each CS solution under stirring for 15min at room temperature resulting in the preparation of CS/TPP in the nano- form.

Preparation of LGE-CSNPs

0.5 mg/ml of Chitosan was dissolved in 2 % acetic acid solution whereas 0.5 mg/ml of TPP was prepared in distilled water. 6

mL of the TPP solution was mixed with 15 mL of the CS solution at room temperature under stirring. CS/TPP concentration was kept at 2.5:1 (v/v) ratio. The LGE-CSNPs were separated from the opalescent suspension by centrifugation at 20000×g for 1 h at 4 °C using a cooling centrifuge. A graphical diagram was made for the extraction and nanoparticle formulation as shown in Figure 1.





The DPPH method (Abd-Elkader et al., 2021) was used to evaluate the ability of LGE and LGE-CSNPs to scavenge free radicals. The percentage of inhibition was calculated using the following equation: Inhibition (%) = $[(\text{Acontrol} - \text{Asample})/(\text{Acontrol})] \times 100$, where Acontrol is the absorbance of the control and Asample is the absorbance of the sample. All measurements were performed in triplicate and averaged. A spectrophotometric technique (Ferreira et al., 2007) was employed to measure the reducing power of LGE and LGE-CSNPs. A standard curve of ascorbic acid (AA) was prepared, and the reducing power was expressed as EC_{50} (mg/mL), which indicates the concentration required for 50% reduction. The hemolytic activity assay was adapted from the method described by Farias et al., (2013). Briefly, the assay was performed in 2 mL microtubes. Hemolysis percentage was calculated as follows: hemolysis (%) = Abstest/AbsPC \times 100, where Abstest is the absorbance at 540 nm of the 1% cell suspension treated with the sample and AbsPC is the absorbance at 540 nm of the 1% cell suspension treated with deionized water (DW).

HPLC fingerprint of lemongrass aqueous extract

The evaluation of the presence of polyphenolic compounds had been achieved using HPLC analysis using HPLC/RP model Hewlett Packard (HP1050) having C18 Alltima column. (**El-Hefny et al., 2023**). Standard polyphenols were used for comparison. polyphenolic compounds were evaluated at 280nm and expressed in μ g/100ml.

Total phenolic and flavonoid contents of LGE

The total phenolic content (TPC) of LGE was carried out according to **Singleton et al., (1999)** using the Folin-Ciocalteu method. Standard curve

of gallic acid (GA) was constructed at the same time. TPC was expressed as GA equivalent (GAE/mg of dry weight). Total flavonoid content (TFC) of LGE was determined by a modified colorimetric method (**Salem et al., 2013**) using (+)-catechin (CA) as standard. TFC was expressed as CA equivalent (CAE/mg of dry weight).

Experimental animals

Thirty -six adult male albino rats weighing between 160 and 180 grams were obtained from the Institute of Graduate Studies and Research located in Alexandria, Egypt. Following a fortnight for acclimatization to their new environment, the animals were housed in six polypropylene cages with bedding made of wood chips, maintained at a temperature of $25\pm3^{\circ}$ C, and artificially lit with a 12-hour cycle of light and dark to prevent chemical contamination. After two weeks of acclimation, the animals were fed a high-fat diet (HF) and kept on a typical laboratory diet for around four weeks. A more efficient method to start establishing insulin resistance, one of the primary characteristics of type 2 diabetes, has been indicated to be the high-fat diet (**Magalhães et al., 2019**).

Experimental diets

While a high-fat diet includes 22% fat (saturated), 48% carbohydrate, and 20% protein, a regular diet has 5% fat, 53% carbohydrate, 23% crude protein, 4% fiber, 7.6% ash, 12.0% moisture, 1.2% calcium, and 0.73% phosphorus and similar for the other elements, together with no limits to water (**Azad and Sulaiman 2020**).

The rats were starved for 12 hours before receiving an injection of streptozotocin (STZ). T2DM was established after 15 minutes of intraperitoneal (i.p.) injection of a single dose of STZ (35 mg/kg BM), recently dissolved in 0.1M citrate buffer (pH = 4.5). (Azad et al., 2020). Days three and seven following injections were used to evaluate fasting blood glucose (FBG), which indicated hyperglycemia. In this study, diabetic rats were defined as those with blood glucose levels (BGL) greater than 250 mg/dL. After two weeks of acclimatization, the study was extended for an additional four weeks. At the conclusion of the trial, the rats were slaughtered (Dawood et al., 2021). To induce complete anesthesia and ensure that the rats had no sensation, anesthesia was provided by isoflurane inhalation followed by cervical dislocation. The present study's methods and processes were approved by the Ethics Committee (IACUC protocol approval code: 81-2B-0223)

Experimental design

Animals were divided into 6 groups each having 6 rats as represented in Table 1.

Animal group	Description
Group 1	Negative control group (4 weeks of regular feed and one cc of sterile saline 0.9% administered orally by gayage tube every day during the
Group I	study period).
Group 2	Positive control group (4 weeks of Diabetic diet).
	Metformin (100 mg/kg Bwt) through intragastric tube for 4 weeks is the
Group 3	reference medication. Before giving rats MET, it was dissolved in
	ddH ₂ O.
Group 4	The treatment group received 1 ml of LGE-CSNPs (0.5 mg/kg Bwt)
Group 4	diluted in sterile saline 0.9% every day for 4 weeks via a gavage tube.
	1 ml of treatment group LGE-CSNPs (1 mg/kg Bwt) diluted in sterile
Group 5	saline 0.9% was administered orally by gavage tube every day for 4
	weeks.
	1 ml of treatment group LGE-CSNPs (5 mg/kg Bwt) diluted in sterile
Group 6	saline 0.9% was administered orally by gavage tube every day for 4
	weeks.

Table 1. Animal grouping used with their treatments.

Blood sample collection

After the rising period of rats, all animals were anesthetized, and blood samples were collected from the retro-orbital plexus (**Sharma et al., 2021**). Tube containing EDTA (with and without anticoagulant) was used in the collection of blood that was used for serum separation. Blood was cooling centrifuged at 1000xg for 10min at 4°C to separate serum. Serum samples were kept in Eppendorf tubes and stored at -20°C to be analyzed. Anesthetized rats were sacrificed after blood was collected, the pancreas was swiftly removed, washed with cold saline. For histological analysis, small portions of the pancreas were removed and preserved in 10% formalin.

Homogenate preparation

To dissect the Pancreas, homogenate (10% w/v) was prepared in 0.1M phosphate buffer (pH 7.4) using Potter Elvehjem type glass Teflon homogenizer and then centrifuged at10,000×g for 20 min at 4°C (**Bisso et al., 1976**).

Determination of oxidant and antioxidant markers

Lipid peroxidation was determined by thiobarbituric reactive substances (TBARS) (μ mol/g protein) in the homogenate according to the method of **Ohkawa et al.**, (1979). Homogenate glutathione peroxidase (GPx) activity (U/g protein) was determined according to the spectrophotometric procedure of Paglia and Valentine (Paglia and Valentine 1967).

Diabetic parameter (HBA1C)

Diabetic parameter (HBA1C) was determined using HBA1C assay kit (Han et al., 2015).

Histopathology

After the rats were sacrificed, tissues were taken from each group's liver and well-maintained in neutral buffered formalin solution (10%). After 24 h (at least), the tissues are dehydrated ascending ethanol gradient, cleaned in xylene, and then fixed in paraffin wax. Tissue segments (3-5 μ thick) were cut and then stained with hematoxylin and eosin (H&E) (**Bancroft et al., 2008**) and subjected to the light microscopic examination for the histopathologic evaluation.

Preparation of functional fermented milk (Rayeb milk) drink supplemented with LGE-CSNPs

Fresh buffalo milk obtained from Faculty of Agriculture farm, Cairo University, Egypt having *L. casei* HQ177095 and *L. paracasei* HQ177096.1 were utilized as fresh activated cultures. These isolates were first activated by growing them MRS broth medium. They were then inoculated in 10% (w/v) sanitized skim milk at 37°C for 24h under microaerophilic conditions. Four portions of the fermented milk drink (Rayeb milk) were made. The produced drinks were placed in 100 mL sterilized glass cups. Three samples of fresh fermented milk were mixed with LGE-CSNPs in the following proportions (0.5, 1, and 5%), and the samples were then kept in a cooling incubator at 5°C for 4 h before sensory evaluation.

Sensory evaluation of fermented milk (Rayeb milk)

Sensory evaluation of fermented milk (Rayeb milk) was tested by 30 staff from the Home Economy Department, Faculty of Education at Alexandria University, Egypt. They were asked to evaluate appearance, colour, taste, flavor, texture and overall acceptability. For evaluating the sample quality, a numerical hedonic scale with a range of 1 to 9 (1 being very bad and 9 excellent) was used (**Abdulghani et al., 2015**). The Food Sensory Laboratory's individual booths were utilized for the evaluation. (Protocol approval number IACUC : 0306305).

Statistical Analysis

The researchers analyzed the collected data using one-way analysis of variance (ANOVA) with Duncan's multiple range test. This analysis was conducted with SPSS® version 16.0 software. A statistically significant difference between groups was considered present if the resulting p-value was less than 0.05 (Steel et al., 1980).

Results and discussion:

Antioxidant activity using DPPH free radical scavenging capacity of LGE and LGE and LGE-CSNPs

Table 2 summarizes the DPPH free radical scavenging capacity of the tested samples. Ascorbic acid (AA), a well-known antioxidant, exhibited the strongest activity with an IC_{50} value of 4.01 µg/mL.

Lemongrass extract (LGE) also demonstrated free radical scavenging ability, but at a lower level (IC₅₀ = 24.28 μ g/mL). Interestingly, lemongrass extract incorporated with chitosan nanoparticles (LGE-CSNPs) showed the most potent activity among the three samples (IC₅₀ = 15.31 μ g/mL). This suggests that the combination of LGE with chitosan nanoparticles may enhance its antioxidant properties. These findings align with previous studies (Cheel et al., 2005; Tavares et al., 2015; Subedi et al., 2014; Figueirinha et al., 2008b), which reported the presence of antioxidant compounds like tannins, phenolic acids, and flavonoids in lemongrass. The dose-dependent activity observed in this study (Subedi et al., 2014) further supports the potential of LGE and LGE-CSNPs as natural antioxidants.

 Table 2.
 The inhibition concentration values DPPH (IC50) value of sample extracts.

Extracts	DPPH (IC50) µg/ml
Ascorbic	$4.01{\pm}0.02^{a}$
Lemongrass	24.28±0.09°
Lemongrass-CS NPs	15.31±0.1 ^b

IC₅₀ (μ g/ml): inhibitory concentrations at which 50% of DPPH radicals are scavenged. Means in the same column followed by different lower-case letters are significantly different (p<0.05).

The reducing power (EC₅₀) of LGE and LGE-CSNPs

Results in Table 3 revealed that LGE had reducing power (EC₅₀) 58.17 mg/mL which was higher than that of LGE-CSNPs and AA (31.28 and 18.24mg/m, respectively). It had been demonstrated that the LGE-CSNPs was more efficient than the LGE. Lemongrass extract exhibited higher contents of phenolic and flavonoid compounds leading to good antioxidant potential by scavenging the free radicals thus having reducing power assay (Den et al., 2020). Mehlous et al., (2020) studied the ferric reducing assay to reduce reactive oxygen species (ROS) using Sacco satureioides revealed and that the EC_{50} value was calyx 0.544 ± 0.006 mg/ml as compared to BHT (0.328 ± 0.005 mg/ml). Some report a positive correlation between reducing power and phenolic content in plants (Zheng et al., 2006). This aligns with the established presence of phenolic compounds in lemongrass, which are known for their reducing properties (Figueirinha et al., 2008a). Table 3. The reducing power (EC₅₀) of LGE and LGE-CS NPs

Extracts	Reducing power EC ₅₀ (mg/ml)		
Ascorbic	18.24±0.04°		
Lemongrass	58.17±0.29ª		
Lemongrass- NPs	31.28±0.14 ^b		

 EC_{50} (mg/ml): effective concentration at which the absorbance is 0.5.Means in the same column followed by different lower-case letters are significantly different (p<0.05)

Haemolytic activity of LGE and LGE-CS NPs

LGE showed the higher haemolytic activity (IC_{50} of 938.21 mg/mL) than LGE-CSNPs (871.42 mg/mL) as represented in Table 4. This finding revealed that LGE-CSNPs' exhibited haemolytic activity more effective that of LGE. The plant extract chitosan combination was tested for primary toxicity using the RBCs haemolysic test, and the findings showed great safety. However, Haggag (**Haggag et al., 2015**) observed that the mixture is not only safe but also has a protective effect against nephrotoxicity. Additionally, it was reported that C. *citratus* extract helped in several medical uses in health and disease (**Nambiar and Matela 2012**).

The Hemolytic assay (1C50) of LGE and LGE-CSI(15			
Extracts	Hemolytic assay		
	IC 50 (mg/ml)		
Lemongrass	938.21 ± 0.74^{a}		
Lemongrass- NPs	871.42±0.54 ^b		

 Table 4. The Hemolytic assay (IC50) of LGE and LGE-CSNPs

 \pm SD is the mean of three replicates. Means in the same raw followed by different lower-case letters are significantly different (p<0.05). IC₅₀ (µg/ml): Extracts concentration that causes 50% haemolysis of RBCs.

High performance liquid chromatography (HPLC) of the aqueous extract of lemongeass

The total phenolic content of lemongrass aqueous extract (LGE) as measured by Folin-Ciocalteu reagent (FCR) was 150, 71±, VA mg GAE/g whereas as the total flavonoid content as measured by the $9^{\Lambda}, 1^{\pm}, 1^{\pm}$ mg CAE/g. On other hand HPLC colorimetric metho was fingerprint study (Table 5 and Figure 2 a &b) revealed that chlorogenic acid (14.56 mg/mL) is the most abundant phenolic compounds followed by ferulic acid (4.56 mg/mL), gallic acid (3.66 mg/mL), and pyrogallol (0.081 mg/mL). Likewise, quercetin (18.25 mg/mL), epicatechin (7.89 mg/mL), chrysoeriol (7.49 mg/mL), and chrysin (5.96 mg/mL) are main flavonoid compounds in LGE. Extracts from C. citratus are composed of several phenolic and flavonoid compounds like ferulic acid, gallic acid, chlorogenic acid, pyrogallol, apigenin, rutin, chrysin, epicatechin, quercetin, chrysoeriol, 7-hydroxyflavanone and naringin. The extract from dry leaves of lemongrass showed the presence of TPC at 41.6 ± 0.05 mg GAE/g freeze-dried extract (Tavares et al., 2015). It was highlighted that the polyphenols from C. citratus, especially chlorogenic acid, are bioactive compounds (Francisco et al., 2013). The most effective antiradical capacity by DPPH. was demonstrated by tannins, phenolic acids (caffeic and p-coumaric acid derivatives), and flavone glycosides derivatives). Isoorientin, (apigenin and luteolin isoscoparin,

swertiajaponin, isoorientin 2 '-O-rhamnoside, orientin, chlorogenic acid, and caffeic acid were isolated from C. citratus extracts and showed free radical scavenging effects (Cheel et al., 2005). It had been reported that LGE possessed free radical-scavenging activities that assisted in decreasing the oxidative damage to the liver caused by cisplatin (Arhoghro and Kpomah 2013). Furthermore, the high flavonoid content and antioxidant properties of LGE may contribute to its renal protective action against gentamicin-induced toxicity (Ullah et al., 2013). It was previously mentioned that the phenolic content of LGE decreased cytokine and nuclear factor-route expression (Francisco et al., 2013) that were responsible for their anti-inflammatory qualities. It has also been observed that LGE therapy reduces the expression of TNF. It has been found that hyperglycaemia-induced inflammation occurs in diabetic cases (Overayo et al., 2021). The activity of α -amylase and α -glucosidase is decreased by treatment with C. citratus extract (Boaduo et al., 2014). Whereas flavonoid, and polyphenolic compounds had hypoglycaemic action (Huang et al., 2015).

Phenolic compound	RT (min)	Concentration (mg/ml)	Phenolic type
Ferulic acid	5.2	4.56	Phenolic
Gallic acid	7.0	3.66	Phenolic
Chlorogenic acid	8.1	14.56	Phenolic
Pyrogallol	13.0	0.081	Phenolic
Apigenin	3.0	3.66	Flavonoid
Rutin	4.0	1.23	Flavonoid
Chrysin	5.0	5.96	Flavonoid
Epicatechin	6.0	7.89	Flavonoid
Quercetin	7.0	18.25	Flavonoid
Chrysoeriol	10.0	7.49	Flavonoid
7-Hydroxyflavanone	12.0	2.14	Flavonoid
Naringin	13.0	2.69	Flavonoid

Table 5. Phenolic and flay	vonoid comp	oounds of LGE	using HPL	C analysis.



Figure 2. HPLC chromatograms of phenolic compounds (a) and flavonoid compounds (b) identified in LGE

Oxidative stress of LGE-CSNPs

TABRAS values in LGE-CS NPs treated rats was assessed. It had been found that TABRAS was lower in the reference drug (MET) group than in the control group. LGE-CSNPs at 5 mg/kg Bwt has the lowest levels of TABRAS. On the other hand, GPx antioxidant enzyme activity was shown to be lowest in the control group, high in the reference medication (MET), and were highest in LGE-CSNPs (5 mg/kg Bwt) and LGE-CSNPs (1 mg/kg Bwt), as represented in Table 6. Lemongrass leaf extract (LGE) in different concentrations were used to overcome the oxidative stress of fibroblasts exposed to H_2O_2 by lowering reactive oxygen species (ROS) levels (**Fitria et al., 2022**). The results indicated that LGE reduced the production of ROS, and this effect was due to the presence of secondary metabolites (phenols and flavonoids) in LGE that act as a non-enzymatic antioxidant that attack and neutralize free radicals. LGEs exhibited DNA protection against free radical generated by H_2O_2 (**Balakrishnan et al., 2014**).

	1 0 1		
Sample code	GPx (U/g protein)	TBARS (µmol/g protein)	
Negative control	45.63±0.18°	11.18±0.01 ^d	
Positive control	31.25 ± 0.12^{f}	22.31±0.04 ^a	
Reference drug (MET)	42.25 ± 0.17^{d}	11.15±0.02 ^e	
LGE-CSNPs (0.5 mg/kg bwt)	35.21±0.16 ^e	14.32±0.03 ^b	
LGE-CSNPs (1 mg/kg bwt)	46.21±0.19 ^b	11.21±0.01°	
LGE-CSNPs (5 mg/kg bwt)	$50.26{\pm}0.17^{a}$	10.51 ± 0.01^{f}	

 Table 6. Antioxidant and oxidant parameters in different studied rat groups

The tables present data as averages \pm SD of ten replicates. Means in the same raw followed by different lower-case letters are significantly different (p<0.05). Means with similar letters are not significantly.

Effect LGE-CSNPs on diabetic parameter (HBA1C)

The HBA1C level of the control positive group was significantly greater than that of the control negative group. In comparison to MET group, HBA1C was lower in the LGE-CSNPs groups, particularly at the 1 and 5 mg/kg Bwt doses (Table 7). In this study, the HBA1C level of the control positive group was significantly greater than that of the control negative group. HBA1C was lower in the LGE-NPs groups than in the Reference drug (MET) group, namely at 1 mg/kg and 5 mg/kg Bwt. Compared to GAE/g freeze-dried extract (Tavares et al., 2015). Collectively, compared to MET, the conventional anti-diabetic drug, LGE-CSNPs had more anti-diabetic effects. It had been observed that oral administration of alcoholic or aqueous extracts of lemongrass to rats with hyperlipidaemia, non-diabetes diabetes, and led to an antihyperlipidemic effect (Ademuyiwa et al., 2015). C. citratus reduces blood sugar and had anti-inflammatory properties, but little is known about the mechanisms underlying these effects and the genes accountable (Ademuyiwa and Grace 2015; Kiani et al., 2022). Rats given alloxan injections, the effects of LGE powder enhanced glucose levels, liver functions, kidney functions, and lipid profile (El-Sayed et al., 2021). Since the islet of Langerhans' alpha and beta cells, respectively, produce glucagon and insulin, the pancreas is necessary for preserving glucose homeostasis and metabolism (Edgerton et al., 2021).

Sample code	HBA1C
Control negative	4.55±0.09 ^e
Control positive	$6.83{\pm}0.05^{a}$
Reference drug (MET)	5.62 ± 0.10^{b}
LGE-CSNPs (0.5mg/kg bwt)	5.66±0.03 ^b
LGE-CSNPs (1mg/kg bwt)	5.39±0.05°
LGE-CSNPs (5mg/kg bwt)	$5.13{\pm}0.07^{d}$

 Table 7. Diabetic parameter (HBA1C) in different studied rat groups

Abbreviations: HBA1C: Gyloslated hemoglobin and MET: Metamorphin. Means in the same raw followed by different lower-case letters are significantly different (p<0.05). Means with similar letters are not significantly.

Histological parameters of Pancreas

The histopathological parameters of Pancreas from 6 animal groups as impacted by various doses of LGE-CSNPs are shown in Figure 3. Negative control group (group1), showed normal histological structure of pancreatic cells, showing normal cells of islets of langerhans in the center (black arrows), embedded in normal surrounding exocrine portion of pancreas (pancreatic acini) (red arrows) (Figure 7A). Positive control group STZ group (group2) exhibited completely degenerated and necrotic islet of Langerhans replaced by cystic structure or completely absent in other parts (black arrows), surrounding exocrine portion of pancreas (pancreatic acini) (red arrows) (Figute 7B). results of group 3(METtreated) revealed normal histological structure of pancreatic cells with normal cells of islets of Langerhans in the center (black arrows) that embedded in normal surrounding exocrine portion of pancreas (pancreatic acini) (red arrows) (Figure 7C). histopathological study of group 4 (LGE-CSNPs (0.5 mg/kg Bwt)) showed degenerated and necrotic effect with shrinkage and reduced cell number islet of Langerhans (black arrows) that embedded in nearly normal surrounding exocrine portion of pancreas (pancreatic acini) (blue arrows) (Figure 7D). On the other hand, rats treated with LGE-CSNPs (1 mg/kg Bwt) exhibited normal histological structure of pancreatic cells with normal cells of islets of langerhans in the center (lack arrows) that embedded in normal surrounding exocrine portion of pancreas (pancreatic acini) (red arrows) were observed (Figure 7E). Moreover, rats treated with LGE-CSNPs (5 mg/kg Bwt) exhibited normal histological structure of pancreatic cells with normal cells of islets of Langerhans in the center (black arrows) that embedded in normal surrounding exocrine portion of pancreas (pancreatic acini) (red arrows) (Figure 7F). That's mean that LGE-CSNPs (1 mg/kg bwt and 5 mg/kg bwt) have an antidiabetic and pancreas to protective effect more than LGE-CSNPs (0.5 mg/kg bwt) and treated group with MET.



Fig. 3. Histological parameters of Pancreas at X40 H&E with stained sections. Where, A: group 1 (Negative control), B: group 2 (Positive control), C: group 3 (Reference with MET)), E: group 4 (LGE-CSNPs 0.5 mg/kg bwt)), F: group 5 (LGE-CSNPs 1 mg/kg bwt)) and F: group 6 (LGE-CSNPs 5 mg/kg bwt)). Sensory evaluation of fermented milk fortified with LGE-CSNPs

According to the results given in Table 8, the addition LGE-CSNPs had positive impact on the organoleptic qualities of basic fermented milk. It scarcely affects anything in terms of appearance flavor, texture, color,

or acceptance in general (Figure 4). Table 8 demonstrated that the overall organoleptic characteristics score (range from 8.33 to 8.77) of the fermented milk didn't differ significantly from any of the fresh treatments. Fresh fermented milk fortified with LGE-CSNPs 0.3% achieved a strong overall score of 8.77. The organoleptic qualities of the base fermented milk (rayeb milk) were changed by the addition of LGE-CSNPs. The effects on appearance, color, flavor, texture, and overall acceptability are all negligible. Due to the larger surface-to-volume ratio of CSNPs, storage stability, and improved hydro-dispersibility, the use of nano-encapsulation as food supplement has been advertised as the most efficient and offered increased loading capacity (Soltanzadeh et al., **2022**). The nanoemulsions have a propensity to encapsulate beneficial compounds, such phytochemicals and antioxidants. as These nanoemulsions are employed in the food-based industries for the regulated release of Flavors components in foods (Guerra-Rosas et al., 2016).

		Sensory attributes					
Samples	Ratio	Appearance	Color	Taste	Flavor	Texture	Overall Acceptability
C.F.M	Control	7.30±0.75	$7.63{\pm}0.49$	8.03±0.41	8.23±0.94	8.71±0.59	8.33±0.81
F.M. LGE- CSNPs	(0.5)	7.37±0.77	7.65±0.62	8.13± 0.72	8.43±0.68	8.57±0.63	8.57±0.63
F.M. LGE- CSNPs	(1%)	7.57±0.63	7.67±0.55	8.17±0.95	8.47±0.68	8.50±0.63	8.61±0.49
F.M. LGE- CSNPs	(5%)	7.73±0.64	7.71±0.47	8.37±0.77	8.67±0.55	8.41±0.86	8.77±0.43
	F	2.39	0.108	1.369	1.80	1.013	2.602
	Р	0.072 ^{ns}	0.955 ^{ns}	0.256 ^{ns}	0.151 ^{ns}	0.389 ^{ns}	0.055 ^{ns}

Table 8	. Sensor	y evaluation	of fermented	milk fortified	with LGE-CSNPs

F.F test One-Way (ANOVA). Values are expressed as mean \pm SD for each fermented milk fortified with LGE-CSNPs. Data was expressed using mean \pm SE. ns: Statistically not significant. C.F.M = Control fermented milk, F.M. LGE-CSNPs = Fermented milk fortified with LGE-CSNPs.



Fig. 4. Sensory evaluation of fermented milk fortified with LGE-CSNPs. (1) C.F.M control, (2) F.M. LGE-CSNPs (0.5%), (3) F.M. LGE-CSNPs (1%) and (4) F.M. LGE-CSNPs (5%)

Conclusion

This study concluded that the aqueous extract of *C. citratus* leaves (LGE) and chitosan nanoparticles loaded with that extract (LGE-CSNPs) exhibit antioxidant, and anti-diabetic properties in addition to their sensory attributes when used as a food supplement for fermented milk. LGE and LGE-CSNPs specifically improved the diabetes mellitus index and hyperglycaemia. The high phenolic and flavonoid contents of *C. citratus* leaves were linked to their strong antioxidant activity, as demonstrated by the experimental study. According to a recent study, using lemongrass or its extract daily can help shield our bodies from oxidative stress and free radical damage. Ultimately, the findings indicate that LGE therapy has an antioxidant impact by lowering lipid peroxidation and raising glutathione peroxidase levels, which improves liver damage and shows that C. *citratus* has a strong hepatoprotective effect.

Ethics approval and consent to participate: The study was authorized by the Ethics Committee for the handling and utilization of animals, organisms and biological cell cultures educational institutions and research in science (Protocol approval number IACUC: 81-2B-0223; and 0306305

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