

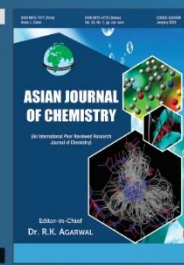


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Biofunctional Potential of Luteolin-Loaded Sodium Alginate–Chitosan Nanoparticles: Antioxidant, Antihyperglycemic and Antiglycation Activities

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The present study reports the *in vitro* antioxidant, enzyme inhibitory, glucose diffusion, yeast-model glucose uptake-related and antiglycation activities of luteolin-loaded sodium alginate–chitosan nanoparticles (LLCNPs). LLCNPs exhibited concentration-dependent DPPH radical-scavenging activity and ferric-reducing antioxidant power (FRAP). In the DPPH assay, LLCNPs showed an IC₅₀ value of 5.95 µg/mL, compared with 6.86 µg/mL for ascorbic acid. The FRAP assay showed a concentration-dependent increase in the ferric reducing activity of LLCNPs, with the highest response observed at 100 µg/mL. LLCNPs also demonstrated concentration-dependent α-amylase and α-glucosidase inhibitory activities. In the glucose diffusion assay, LLCNPs reduced glucose diffusion across the dialysis membrane under the experimental conditions. The yeast-model assay demonstrated glucose uptake-related activity as indicated by a reduction in residual glucose in the presence of LLCNPs. LLCNPs also exhibited antiglycation activity across the tested concentration range. Comparative evaluation with luteolin, blank nanoparticles and reference compounds demonstrated differences among the tested formulations. These findings confirm that LLCNPs exhibit several measurable *in vitro* biofunctional activities.

Keywords: Luteolin, Sodium alginate-chitosan, Antidiabetic activity, Antioxidant activity, Anti-glycation.

INTRODUCTION

In recent years, natural products and medicinal plants have attracted considerable attention as potential sources of antidiabetic agents due to their therapeutic efficacy, accessibility and relatively low toxicity [1]. Plant-derived phytochemicals, particularly polyphenols and flavonoids, possess antioxidant and antihyperglycemic properties. These compounds can neutralize reactive oxygen species (ROS) and free radicals, thereby reducing oxidative stress, a major factor involved in the development and progression of diabetes and its complications [2,3]. Medicinal plants further provide a diverse source of structurally distinct compounds that have contributed to modern drug discovery and pharmaceutical development [4].

Despite advances in modern medicine, traditional herbal remedies remain important in healthcare systems worldwide. More than 80% of the population in developing countries is estimated to rely on traditional medicine for primary healthcare, while the use of complementary and alternative medicine has increased in developed countries. Nearly one-quarter

of pharmaceutical drugs are derived directly or indirectly from plant sources. These facts support the continued investigation of plant-derived bioactive molecules for the prevention and management of chronic diseases such as diabetes mellitus [5].

Among the phytochemicals investigated for antidiabetic applications, luteolin (3',4',5,7-tetrahydroxyflavone) has been emerged as a promising candidate. This naturally occurring flavonoid is widely distributed in medicinal plants, fruits, vegetables and nutraceutical products. Luteolin possesses antioxidant, anti-inflammatory, antimicrobial, anticancer and cytoprotective activities. Its effects on oxidative stress, inflammatory pathways, glucose metabolism and cellular signalling make it a potential candidate for the management of chronic disorders, including diabetes, cardiovascular diseases and hepatic dysfunction [6-8].

The clinical application of luteolin is limited by poor aqueous solubility, low oral bioavailability, rapid metabolism and inadequate accumulation at target tissues, which can reduce its therapeutic efficacy and hinder pharmaceutical

translation [9]. Nanotechnology-based delivery systems offer an approach to address these limitations by improving solubility, physico-chemical stability, drug release, cellular uptake and tissue delivery. Among these systems, biodegradable polymeric nanoparticles are attractive due to their biocompatibility and biodegradability. Chitosan, a naturally occurring cationic polysaccharide, is widely used as a nanoparticle carrier owing to its low toxicity, mucoadhesive properties and ability to enhance drug absorption and bioavailability. Nanoencapsulation can improve the stability and biological performance of phytochemicals while overcoming limitations associated with conventional formulations [10-12].

Oxidative stress plays an important role in diabetes, while luteolin possesses several pharmacological properties that may be beneficial in its management. Therefore, incorporating luteolin into nanoparticle-based delivery systems could be a promising approach to enhance its antidiabetic activity [13,14]. In this study, luteolin loaded sodium alginate–chitosan nanoparticles (LLCNPs) were evaluated for antioxidant, antidiabetic and antiglycation activities using complementary *in vitro* models. The biological performance of LLCNPs was compared with free luteolin and blank sodium alginate–chitosan nanoparticles, allowing the effects of luteolin loading to be distinguished from those of the polymeric carrier. This comparative approach provides an integrated *in vitro* assessment of the developed formulation and its potential as a nano-based delivery system for luteolin.

EXPERIMENTAL

Chitosan (m.w.: 222 kDa), sodium alginate with a β -D-mannuronic acid (M)/ α -L-guluronic acid (G) ratio of 64.5:35.5 and luteolin (LUT) were purchased from Sigma-Aldrich (USA). Dialysis membranes were obtained from HiMedia Laboratories, India. All other chemicals and solvents employed in this study were of analytical reagent grade and used without further purification. Distilled water was used throughout the experimental procedures.

Synthesis of blank sodium alginate-chitosan nanoparticles via ionotropic gelation: Blank sodium alginate-chitosan nanoparticles were prepared using a modified two-step ionotropic gelation technique based on the method previously described earlier [15]. Sodium alginate and calcium chloride solutions were prepared in distilled water. The pH of the sodium alginate solution was adjusted to 5.1 using HCl, while chitosan was dissolved in 1% (v/v) acetic acid solution and subsequently adjusted to pH 5.4 with NaOH solution. Briefly, 2 mL of calcium chloride solution (3.35 mg/mL) was added dropwise to the sodium alginate solution under continuous magnetic stirring at 1200 rpm to facilitate the formation of a calcium alginate pre-gel network. The resulting dispersion was stirred for 30 min to ensure complete ionic crosslinking between calcium ions and alginate chains. Subsequently, 4 mL of chitosan solution (0.8 mg/mL) was added gradually to the pre-gel system under constant stirring, allowing electrostatic interactions between the positively charged chitosan and negatively charged alginate molecules, thereby promoting the nanoparticle formation.

The resulting opalescent nanoparticle suspension was maintained under gentle stirring and allowed to equilibrate

overnight at room temperature to achieve complete stabilization and a homogeneous particle size distribution. The prepared blank chitosan–alginate nanoparticles were subsequently used for further formulation studies.

Fabrication of luteolin-loaded sodium alginate-chitosan nanoparticles (LLCNPs): Luteolin-loaded sodium alginate-chitosan nanoparticles (LLCNPs) were fabricated using the ionotropic gelation method described for the preparation of blank nanoparticles, with minor modifications [16]. Briefly, 300 μ L of luteolin solution (1.105 mg/mL), prepared in a dehydrated ethanol–water mixture (1:1, v/v), was incorporated into the calcium chloride solution prior to nanoparticle synthesis. The luteolin-containing calcium chloride solution was then added dropwise to the sodium alginate solution under continuous magnetic stirring to facilitate the formation of a luteolin-entrapped calcium alginate pre-gel network.

Following pre-gel formation, the dispersion was stirred for 30 min to ensure uniform distribution and efficient entrapment of luteolin within the polymeric matrix. Subsequently, chitosan solution was added gradually under constant stirring, promoting electrostatic interactions between the oppositely charged biopolymers and resulting in the formation of luteolin loaded nanoparticles. The resulting nanoparticle suspension was allowed to equilibrate overnight at room temperature to achieve complete stabilization and uniform particle size distribution.

Antioxidant assays

DPPH radical scavenging assay: The antioxidant activity of the samples was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay [10]. A 0.1 mM DPPH solution was prepared by dissolving 2 mg of DPPH in 30 mL of ethanol. Briefly, 40 μ L of sample extract or nanoparticle suspension at different concentrations was mixed with 3 mL of DPPH solution and incubated in the dark at room temperature for 30 min. Ethanol and ascorbic acid were used as the negative and positive controls, respectively. The reduction of DPPH radicals was indicated by a colour change from purple to yellow and the absorbance was measured at 517 nm using a UV-Visible spectrophotometer. The percentage of radical scavenging activity was calculated as:

$$\text{Inhibition (\%)} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100$$

This assay assesses the free radical scavenging capacity of the samples through their hydrogen-donating ability.

Ferric reducing antioxidant power (FRAP) assay: The ferric reducing antioxidant power (FRAP) of the samples was determined according to the reported method [17] with slight modifications. The FRAP reagent was freshly prepared by mixing 300 mM sodium acetate buffer (pH 3.6), 10 mM TPTZ solution in 40 mM HCl and 20 mM $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ in a ratio of 10:1:1. Sample extracts at concentrations ranging from 20–100 μ g/mL were added to 3 mL of FRAP reagent and incubated at 37 °C for 30 min. The absorbance was then recorded at 593 nm using a UV-Visible spectrophotometer. Ascorbic acid was used as the reference standard and calculated using the following equation:

$$\text{Inhibition (antioxidant) (\%)} = \frac{\text{Abs}_{\text{sample}} - \text{Abs}_{\text{control}}}{\text{Abs}_{\text{sample}}} \times 100$$

In vitro antidiabetic activity

α -Amylase inhibition assay: The α -amylase inhibitory activity of the samples was determined using a modified method [18]. Briefly, 100 μL of α -amylase solution (0.2 mg/mL; 2.6 U/mL) was mixed with 100 μL of sample at different concentrations prepared in 0.02 M phosphate buffer (pH 6.6) and pre-incubated for 15 min at room temperature. Subsequently, 100 μL of 1% starch solution was added to initiate the reaction, followed by incubation for 30 min. The reaction was terminated by adding 200 μL of dinitrosalicylic acid (DNS) reagent and 500 μL of phosphate buffer (0.02 M, pH 6.9). The mixture was heated at 100 $^{\circ}\text{C}$ for 10 min, cooled to room temperature and the absorbance was measured at 540 nm using a UV-Visible spectrophotometer. The percentage inhibition of α -amylase activity was calculated as:

$$\text{Inhibition (\%)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

This assay evaluates the ability of the samples to inhibit starch hydrolysis and reduce glucose release.

α -Glucosidase inhibition assay: The α -glucosidase inhibitory activity was assessed according to the reported method [19]. The reaction mixture consisted of 50 μL phosphate buffer (50 mM, pH 6.8), 10 μL α -glucosidase enzyme and 20 μL of sample at various concentrations. After pre-incubation at 37 $^{\circ}\text{C}$ for 15 min, 20 μL of *p*-nitrophenyl- α -D-glucopyranoside (PNPG) substrate was added and incubated for 30 min at 37 $^{\circ}\text{C}$. The reaction was stopped by adding 50 μL of 0.1 M Na_2CO_3 and the absorbance of the released *p*-nitrophenol was measured at 405 nm. Metformin was used as the reference drug. The percentage inhibition was calculated using the following equation:

$$\text{Inhibition (\%)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

The assay was used to evaluate the ability of luteolin, LLCNPs and the corresponding controls to inhibit α -glucosidase activity under the experimental conditions.

Effect of encapsulated luteolin on *in vitro* glucose diffusion: The dialysis-based glucose diffusion assay was used to evaluate the ability of the test formulations to modulate glucose diffusion across a dialysis membrane under *in vitro* experimental conditions [20]. Briefly, a dialysis tube containing 3 mL of glucose solution (22 mmol L^{-1} in 0.15 mol L^{-1} NaCl) and 1 mL of encapsulated luteolin sample (5 mg/kg) was sealed and immersed in 45 mL of 0.15 mol L^{-1} NaCl solution. Distilled water served as the control. The system was incubated in a shaking water bath at 37 $^{\circ}\text{C}$ and glucose concentrations in the external medium were determined at 30, 60, 120 and 180 min using a glucose oxidase–peroxidase assay kit. The assay provides a preliminary assessment of changes in glucose diffusion and does not directly represent intestinal glucose absorption *in vivo*.

Glucose uptake by yeast cells: Glucose uptake by yeast cells was determined following the method described elsewhere [21]. Commercial baker's yeast was repeatedly centrifuged at 3,000 $\times$ g for 5 min until a clear supernatant was obtained and a 10% (v/v) yeast suspension was prepared in distilled water. Sample solutions at different concentrations (20–100 $\mu\text{g/mL}$) were mixed with 1 mL of glucose solution (5, 10 or 25 mM) and pre-incubated at 37 $^{\circ}\text{C}$ for 10 min. Subsequently, 100 μL of yeast suspension was added, vortexed and incubated at 37 $^{\circ}\text{C}$ for 60 min. The mixtures were centrifuged at 2,500 $\times$ g for 5 min and the residual glucose concentration in the supernatant was measured. Metformin was used as the reference standard. The percentage glucose uptake was calculated based on the reduction in residual glucose relative to the control using the following equation:

$$\text{Glucose uptake (\%)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

where A_{control} represents the absorbance of the control reaction and A_{sample} represents the absorbance of the test sample. A lower residual glucose concentration in the sample relative to the control was considered indicative of greater glucose uptake by the yeast cells. All experiments were performed in triplicate.

Antiglycation assay: The antiglycation activity of the samples was evaluated using the bovine serum albumin (BSA) glucose/fructose glycation model with minor modifications [22]. Briefly, BSA (1 mg/mL) was incubated with 0.25 M glucose and 0.25 M fructose in phosphate-buffered saline (PBS; 0.1 M, pH 7.4) at 50 $^{\circ}\text{C}$ for 4 days in the dark. Sample solutions prepared in 50% DMSO were added to the reaction mixture prior to incubation. The formation of advanced glycation end products (AGEs) was quantified by measuring fluorescence intensity at an excitation wavelength of 335 nm and an emission wavelength of 385 nm. The percentage inhibition of AGE formation was calculated using the following equation:

$$\text{AGE inhibition (\%)} = \frac{(F_{\text{control}} - F_{\text{control blank}}) - (F_{\text{sample}} - F_{\text{sample blank}})}{(F_{\text{control}} - F_{\text{control blank}})} \times 100$$

where $(F_{\text{control}} - F_{\text{control blank}})$ is the difference between the fluorescent intensity of BSA incubated with or without glucose and fructose and $(F_{\text{sample}} - F_{\text{sample blank}})$ is the difference between the fluorescent intensity of BSA and sugars incubated with or without plant extracts. This assay evaluates the ability of the samples to inhibit non-enzymatic protein glycation, a key process involved in the development of diabetic complications.

Statistical analysis: All experiments were performed in triplicate and the results were expressed as mean $\pm$ standard error of the mean (SEM). Statistical analyses were carried out.

RESULTS AND DISCUSSION

Antioxidant activity of luteolin-loaded sodium alginate-chitosan nanoparticles using DPPH assay: As shown in Fig. 1, all tested formulations exhibited a concentration-dependent increase in DPPH radical scavenging activity over the concentration range of 20–100 $\mu\text{g/mL}$. Ascorbic acid

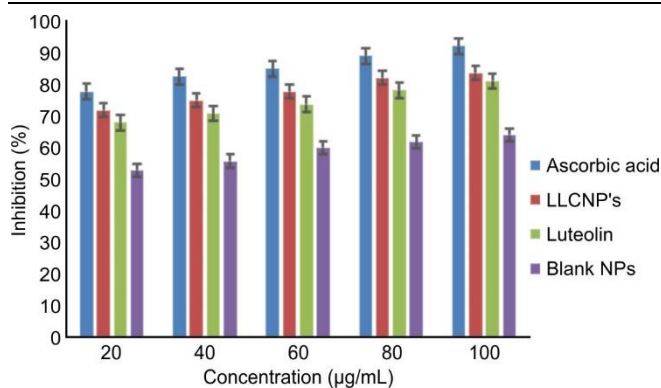


Fig. 1. DPPH radical scavenging activity of LLCNPs, ascorbic acid (std.), luteolin and blank nanoparticles

exhibited the highest radical scavenging activity at all tested concentrations, with inhibition increasing from approximately 77% at 20 µg/mL to 92% at 100 µg/mL. LLCNPs also demonstrated a progressive increase in DPPH inhibition, from approximately 71% to 83% across the corresponding concentration range.

Interestingly, LLCNPs exhibited higher DPPH radical scavenging activity than luteolin at all tested concentrations. Luteolin showed 67–80% inhibition, whereas LLCNPs showed 71–83% inhibition from 20 to 100 µg/mL. This difference suggests that incorporation of luteolin into the chitosan–alginate nanoparticle system may improve its availability for interaction with DPPH radicals under the experimental conditions. Blank nanoparticles showed comparatively lower radical scavenging activity (~52–63%), while the higher activity of LLCNPs points to a contribution from luteolin beyond the nanoparticle matrix.

Similar findings have been reported for other flavonoid-based nanoparticle systems. Quercetin-loaded alginate–chitosan nanoparticles (ALG/CSNPs) showed higher DPPH radical-scavenging activity than free quercetin. At 100 µg/mL, quercetin-loaded ALG/CSNPs produced 82.21% inhibition, compared with 78.13% for free quercetin, while the reference antioxidant ascorbic acid showed 87.2% inhibition under the same conditions [23]. Silver nanoparticles (AgNPs), gold nanoparticles (AuNPs) and quercetin have similarly been reported to exhibit concentration-dependent antioxidant activity, with AgNPs showing the highest scavenging activity. At 250 µg/mL, AgNPs produced 89.59% DPPH inhibition, compared with 94.9% for ascorbic acid, 83.32% for AuNPs and 73.22% for quercetin [24]. The corresponding IC_{50} values were 100.4, 120.8 and 146.36 µg/mL for AgNPs, AuNPs and quercetin, respectively, which further support the superior radical scavenging activity of AgNPs.

The IC_{50} values calculated from the concentration–response data were 5.95 µg/mL for LLCNPs and 6.86 µg/mL for ascorbic acid, respectively. The lower IC_{50} value obtained for LLCNPs indicates a higher DPPH radical scavenging capacity on the basis of the calculated IC_{50} parameter. The concentration-dependent response and enhanced activity of LLCNPs relative to luteolin support their *in vitro* antioxidant potential.

FRAP assay of LLCNPs: The FRAP of ascorbic acid, LLCNPs, luteolin and blank nanoparticles is shown in Fig. 2. All tested samples exhibited concentration-dependent ferric

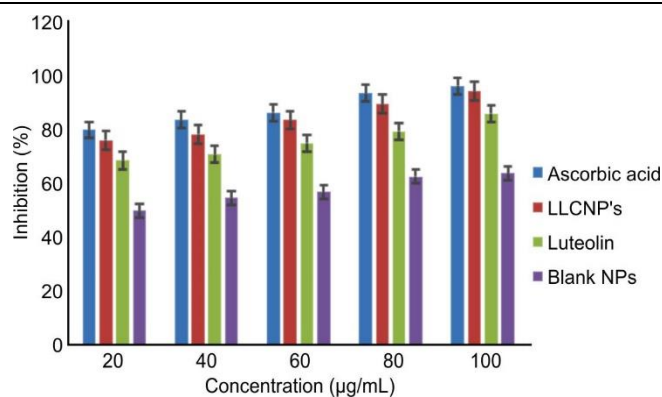


Fig. 2. FRAP activity of LLCNPs, ascorbic acid (std.), luteolin and blank nanoparticles

reducing activity, with the reducing capacity increasing progressively as the concentration increased from 20 to 100 µg/mL. Among the tested samples, ascorbic acid showed the highest reducing activity, followed by LLCNPs, luteolin and blank nanoparticles. At 100 µg/mL, LLCNPs exhibited ~94% reducing activity compared with ~97% for ascorbic acid, whereas luteolin and blank nanoparticles showed ~86% and 64% activity, respectively.

Comparable antioxidant activity has been reported for plant extracts and luteolin-based delivery systems. Rafei *et al.* [25] reported substantial antioxidant activity for *Rhododendron arboreum* extracts in DPPH and FRAP assays, with lower IC_{50} values associated with stronger antioxidant activity. The methyl acetate extract showed the highest ferric-reducing capacity (IC_{50} = 163.6 µg/mL), whereas the methanolic extract exhibited the strongest DPPH scavenging activity. A whey protein isolate–luteolin liposomal formulation similarly showed higher FRAP activity (82.86 ± 1.42) than LUT-Lips (74.78 ± 1.30), which was attributed to improved dispersion and favourable interactions between whey protein isolate (WPI) and luteolin [26]. Hydrogen bonding between WPI and the hydroxyl groups of luteolin may facilitate electron donation to the Fe^{3+} –TPTZ complex, while reduced steric hindrance around the B-ring hydroxyl groups could further improve ferric-ion reduction.

In present study, LLCNPs showed a concentration-dependent increase in ferric-reducing activity, reaching 95.32 at 100 µg/mL, which close to the ascorbic acid value of 97.23 µg/mL. The superior reducing activity of LLCNPs than free luteolin suggests that nanoencapsulation preserved the electron donating capacity of luteolin and may have improved its dispersion in the assay medium. This activity is primarily related to the redox-active hydroxyl groups of luteolin, while the sodium alginate–chitosan matrix may contribute to the measured response. The FRAP results reflect reducing capacity under the experimental conditions and should not be interpreted as direct evidence of enhanced bioavailability or *in vivo* antioxidant efficacy.

Antidiabetic activity: The α -amylase inhibitory activity of luteolin-loaded sodium alginate–chitosan nanoparticles (LLCNPs) was evaluated and the results are shown in Fig. 3. LLCNPs exhibited significant concentration-dependent inhibition of α -amylase activity. The observed inhibition demon-

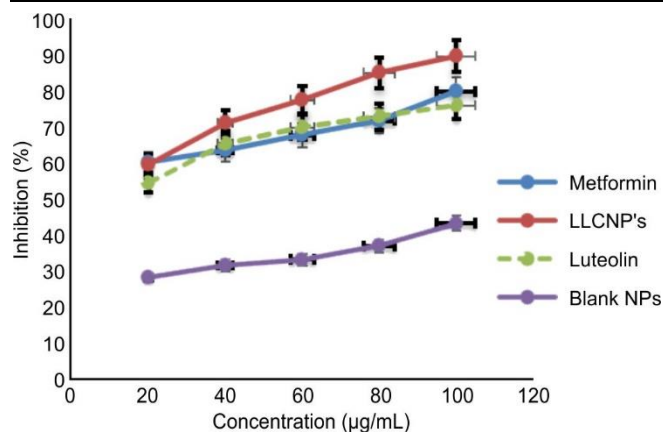


Fig. 3. α -Amylase inhibitory activity of LLCNPs, metformin (std.), luteolin and blank nanoparticles

strates the ability of LLCNPs to inhibit α -amylase under the *in vitro* experimental conditions. LLCNPs showed higher inhibitory activity than luteolin and blank nanoparticles across the tested concentrations, while the activity was also comparable to or higher than that of metformin at high concentrations. Luteolin exhibited a similar concentration-dependent pattern but showed lower inhibition than LLCNPs, whereas blank nanoparticles displayed comparatively lower inhibitory activity throughout the assay.

Several plant extracts have also been investigated for their ability to inhibit α -amylase and α -glucosidase. Yadav *et al.* [27] reported that *Neolamarckia cadamba* methanolic crude extract (NMCA) inhibited both enzymes, with IC_{50} values of 37.23 ± 1.49 and 38.26 ± 1.72 μ M, respectively, approaching the activity of acarbose. Another study [28] found that the *n*-butanol extract had the strongest α -amylase inhibitory activity among the tested fractions ($IC_{50} = 190.0 \pm 70.0$ μ g/mL), followed by the ethanol and ethyl acetate extracts, whereas the petroleum ether extract showed no inhibition. All extracts were less potent than acarbose ($IC_{50} = 1.7 \pm 0.6$ μ g/mL).

In this study, LLCNPs showed concentration-dependent α -amylase inhibition, reaching ~90% at 100 μ g/mL vs. 80% for metformin, 76% for luteolin and 43% for blank nanoparticles. The enhanced response may reflect the intrinsic enzyme-inhibitory properties of luteolin together with improved dispersion of the compound within the nanoparticle system. The hydroxyl groups and aromatic structure of luteolin may favour hydrogen-bonding and hydrophobic interactions with α -amylase. Nanoencapsulation may further improve contact between luteolin and the enzyme in the assay medium. These results support the *in vitro* α -amylase inhibitory activity of LLCNPs and provide a basis for further mechanistic investigation [29].

α -Glucosidase inhibitory activity: The α -glucosidase inhibitory activity of luteolin-loaded sodium alginate-chitosan nanoparticles (LLCNPs) was evaluated in comparison with metformin, luteolin and blank nanoparticles. As shown in Fig. 4, LLCNPs exhibited a concentration-dependent increase in α -glucosidase inhibitory activity, with inhibition progressively increasing from the lowest to the highest tested concentration. Across the tested concentrations, LLCNPs showed higher

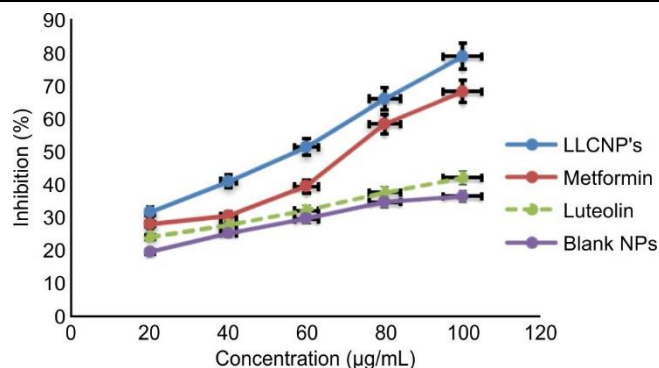


Fig. 4. α -glucosidase inhibitory activity of LLCNPs, metformin (std.), luteolin and blank nanoparticles

inhibitory activity than metformin, luteolin and blank nanoparticles. Luteolin exhibited moderate inhibitory activity, whereas blank nanoparticles showed comparatively lower inhibition throughout the concentration range. The higher inhibitory activity observed with LLCNPs compared with luteolin and blank nanoparticles suggests that the nanoparticle formulation contributed to the α -glucosidase inhibitory effect.

Previous studies have established the α -glucosidase inhibitory activity of luteolin and other plant-derived compounds. Luteolin inhibited α -glucosidase in a concentration-dependent manner at 5–50 μ M, with an IC_{50} of 16.2 μ M ($-\log IC_{50} = 4.79 \pm 0.03$), whereas vernodalol showed no significant inhibition [26]. Epicatechin and epiafzelechin similarly exhibited α -glucosidase inhibition, with IC_{50} values of 5.72 ± 2.7 and 7.64 ± 37.5 μ g/mL, respectively, although both were less potent than acarbose ($IC_{50} = 1.52 \pm 0.05$ μ g/mL); lupeol showed minimal activity ($IC_{50} > 1000$ μ g/mL) [30].

In this study, LLCNPs showed an IC_{50} of 54.35 μ g/mL, compared with 70.09 μ g/mL for metformin, which shows the superior α -glucosidase inhibition under the tested conditions. The enhanced activity of LLCNPs relative to free luteolin may be related to improved dispersion and enzyme interaction following nanoencapsulation. Activity observed with blank nanoparticles suggests that the chitosan-based matrix may contribute to the measured response. LLCNPs therefore exhibited concentration-dependent α -glucosidase inhibition, which support further investigation of this formulation for *in vitro* modulation of carbohydrate-digesting enzymes.

Effect of LLCNPs on *in vitro* glucose diffusion: The effect of LLCNPs on glucose diffusion was evaluated using a dialysis membrane model, with metformin as the reference drug. Dialysis bags containing glucose and the test samples were immersed in NaCl solution and glucose diffusion into the external medium was measured at 30, 60, 90, 120 and 180 min using the glucose oxidase method [21].

Table-1 presents the effect of LLCNPs, metformin, luteolin and blank nanoparticles on glucose diffusion across a dialysis membrane at different time intervals. LLCNPs reduced glucose diffusion compared with the control at 30–180 min, with the lowest external glucose concentration observed at 60 min (51.2 ± 0.5 mM), which suggest a retardant effect on glucose transport. The effect was comparable to metformin at later time points, while luteolin and blank nanoparticles exhibit higher diffusion retardation throughout the assay.

TABLE-1
EFFECTS OF LLCNPS ON GLUCOSE DIFFUSION *in vitro*

Sample	Glucose concentration in external solution at different time intervals (mM)				
	30 min	60 min	90 min	120 min	180 min
Control	57.4 ± 0.6	58.6 ± 0.6	68.8 ± 0.5	86.6 ± 0.8	98.4 ± 0.9
LLCNPs	52.6 ± 0.3	51.2 ± 0.5	67.3 ± 0.3	82.9 ± 0.4	94.7 ± 0.3
Metformin	49.1 ± 0.3	52.9 ± 0.5	66.7 ± 0.8	82.4 ± 0.2	95.4 ± 0.3
Luteolin	42.3 ± 0.2	43.5 ± 0.3	49.8 ± 0.5	51.3 ± 0.2	56.4 ± 0.6
Blank NPs	32.8 ± 0.7	35.1 ± 0.8	37.7 ± 0.5	39.4 ± 0.3	41.8 ± 0.2

Evidence from previous work supports the use of dialysis membrane models for assessing glucose diffusion. Ansari *et al.* [28] reported that ethanolic extract of *Camellia sinensis* reduced glucose diffusion by 7-27% at 0.2-25 mg/mL after 24 h, with significance levels of $p < 0.05-0.001$, which support its potential to delay glucose absorption and influence post-prandial glycemic response. In the present study, glucose concentration in the external medium increased with time in all groups, while the extent of diffusion differed among treatments. LLCNPs maintained lower glucose concentrations than the control at all time points, which reflect the reduced glucose diffusion across the dialysis membrane. Metformin produced a similar pattern, whereas luteolin caused a greater reduction. Blank nanoparticles produced the lowest glucose concentrations, which is due to the contribution from the chitosan-alginate matrix itself. The difference between LLCNPs and free luteolin may relate to changes in diffusion characteristics following nanoencapsulation. These findings support the capability of LLCNPs to modulate glucose diffusion under the present *in vitro* conditions, although this model does not directly establish intestinal glucose absorption or *in vivo* glycemic effects.

Effect of LLCNPs on glucose uptake by yeast cells:

The glucose uptake assay using *Saccharomyces cerevisiae* was performed to evaluate the effect of LLCNPs on cellular glucose utilization. Yeast cells possess membrane-associated glucose transport systems that facilitate carrier-mediated glucose uptake, making this model a widely accepted *in vitro* method for screening antidiabetic activity [31]. Enhanced glucose uptake reflects improved glucose utilization and may indicate potential glycemic regulatory effects. In this study, glucose uptake was evaluated based on the reduction in residual glucose concentration relative to the control.

As shown in Fig. 5a-c, LLCNPs enhanced glucose uptake in a concentration-dependent manner across 5, 10 and 25 mM glucose conditions. Increasing the LLCNP concentration from 20 to 100 µg/mL progressively increased glucose uptake, with the highest response at 100 µg/mL approaching that of metformin and exceeding free luteolin. Previous studies [32] have reported similar glucose-modulating effects for plant-derived agents. The ethyl acetate fraction of *Jasminum officinale* showed glucose uptake inhibition of $17.40 \pm 0.04\%$ to $74.36 \pm 0.01\%$, while methanolic *C. angustifolia* rhizome extract enhanced glucose uptake in yeast cells through facilitated glucose transport and utilization, although less effectively than acarbose [33].

Glucose concentration affected the extent of uptake, with the highest response at 5 mM, followed by 10 and 25 mM. LLCNP-treated cells consistently exhibited higher glucose uptake than luteolin at all tested concentrations, which support a contribution from nanoencapsulation. Improved dispersibility, cellular interaction, surface area and sustained luteolin release may account for this response. The findings support the ability of LLCNPs to enhance glucose uptake in this yeast-cell model, although the results should not be interpreted directly as evidence of improved peripheral glucose utilization or therapeutic efficacy *in vivo*.

Antiglycation activity: The antiglycation potential of LLCNPs was evaluated using the BSA-glucose/fructose glycation model, with metformin used as the reference standard. Luteolin and blank chitosan-alginate nanoparticles were also evaluated under the same experimental conditions to determine the contribution of the free drug and polymeric matrix, respectively. As shown in Fig. 6, LLCNPs exhibited inhibition of AGE formation across the tested concentration range of 20-100 µg/mL. AGE formation was progressively

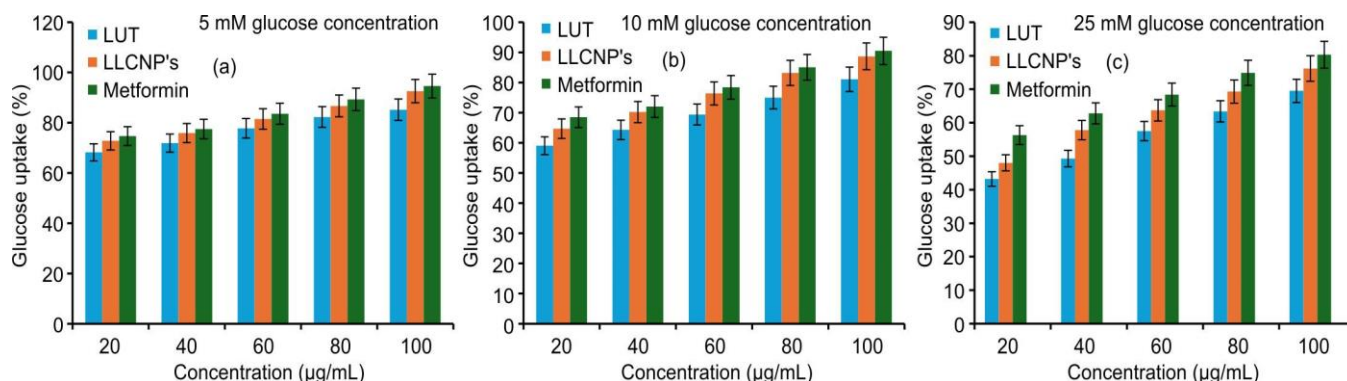


Fig. 5. Effect of luteolin (LUT), luteolin-loaded sodium alginate-chitosan nanoparticles (LLCNPs) and metformin on glucose uptake activity at different concentrations: (a) 5 mM, (b) 10 mM and (c) 25 mM

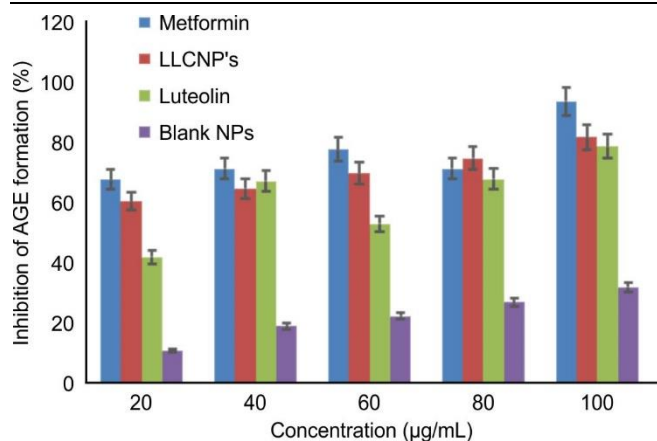


Fig. 6. Antiglycation activity of LLCNPs and metformin (std.)

inhibited with increasing concentration. LLCNPs exhibited greater inhibitory activity than luteolin and blank nanoparticles, whereas metformin remained more effective at the tested concentrations.

Previous studies have reported antiglycation activity for several flavonoids. Quercetin inhibited AGE formation in oxidative and non-oxidative models, with IC_{50} values of 0.051 and 0.040 mg/mL, respectively, while apigenin showed an IC_{50} of 0.45 mg/mL in the non-oxidative model. Juglone exhibited IC_{50} values of 0.11 and 0.06 mg/mL in oxidative and non-oxidative systems, respectively [34]. These findings provide support for the antiglycation activity observed with flavonoid-based compounds. The activity of LLCNPs may be related to the antioxidant and reactive carbonyl-scavenging properties of luteolin, which can interfere with glycation and glycoxidation reactions. Incorporation into the sodium alginate–chitosan matrix may further alter luteolin dispersion and release characteristics. The specific contribution of nanoencapsulation, though, cannot be established from this assay alone and requires direct comparison with free luteolin under identical conditions.

Conclusion

The present study established the successful development and *in vitro* evaluation of luteolin-loaded sodium alginate–chitosan nanoparticles (LLCNPs). The formulation exhibited antioxidant activity in DPPH and ferric-reducing assays, inhibited α -amylase and α -glucosidase, reduced glucose diffusion across a dialysis membrane, enhanced glucose uptake in the *Saccharomyces cerevisiae* model, and inhibited AGE formation in the BSA–fructose assay. Comparisons with free luteolin, blank nanoparticles and reference compounds revealed distinct biological responses, with contributions from both luteolin and the nanoparticle matrix. The results provide preliminary *in vitro* evidence for the antioxidant, enzyme-inhibitory, glucose diffusion-modulating, glucose uptake-related and antiglycation properties of LLCNPs. The dialysis and yeast models are simplified experimental systems and cannot establish delayed intestinal glucose absorption or enhanced peripheral glucose utilization *in vivo*. Changes in bioavailability, stability or therapeutic efficacy following nanoencapsulation were not directly assessed. Mechanistic, pharmacokinetic and *in vivo* studies are needed to define the role of the

sodium alginate–chitosan carrier and establish the biological relevance and therapeutic potential of LLCNPs.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

DECLARATION OF AI-ASSISTED TECHNOLOGIES

During the preparation of this manuscript, the authors used an AI-assisted tool(s) to improve the language and no data, results or scientific conclusions were generated by AI. All analyses, interpretations and conclusions are the authors' own. The authors reviewed and edited the content and take full responsibility for the published work.

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