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Pharmacological Evaluation of *Curcuma longa* in Glucotoxicity Through Antiglycation, Antioxidant, Antidiabetic and Molecular Docking Studies

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Abstract

Diabetes mellitus, which is characterized by hyperglycemia and glucotoxicity, leads to severe metabolic disturbances and complications driven by the accumulation of advanced glycation end products (AGEs). Several medicinal plants, including *Curcuma longa,* have been used traditionally to alleviate the inflammation and wound healing associated with these chronic disorders. However, scientific evidence is scarce regarding the assessment of the potential of *Curcuma longa* in glucotoxicity. Therefore, this study has been designed to evaluate the effect of *Curcuma longa* extract (CLE) in mitigating GO (glyoxal) derived AGEs-induced glucotoxicity through in vitro and in vivo studies, alongside molecular docking analysis. Cytotoxicity assay results suggested the safety doses of CLE, and then the anti-glycation activity was assessed through the GO-AGEs inhibition assay, revealing the significant inhibitory effect of CLE at both low and high doses. Mentionable that, our in vivo studies data from a diabetic mice model strongly supported the anti-diabetic efficacy of CLE through significant reductions in blood glucose levels. Additionally, CLE has exhibited potent antioxidant properties, confirmed by DPPH assays. Besides that, the presence of curcumin has also been confirmed by UV/Visible spectrophotometry through the curcumin percentage and color value determination method. Additionally, molecular docking studies also supported the binding affinities of curcumin (the major bioactive compound of CLE) with different proteins of RAGE signaling pathways. Overall, this comprehensive study provides scientific validation for the use of CLE as a natural therapeutic agent in diabetes targeting glucotoxicity and offers a promising alternative for reducing the burden of diabetes-induced complications.

Key words: *Curcuma longa;* Curcumin; Diabetes mellitus; Glucotoxicity; Advanced glycation end products (AGEs).

Introduction

Diabetes mellitus (DM) is considered as a complex and chronic metabolic disorder, which is characterized by persistent hyperglycemia resulting from abnormalities in insulin secretion, insulin action, or both. This condition has become a significant global health issue, given its increasing prevalence and the severe consequences, such as cardiovascular diseases, neuropathy, kidney damage, and vision problems (Maessen *et al.*, 2015; Talukder and Hossain, 2020). Primarily, type 1 diabetes (T1D) results from the autoimmune destruction of pancreatic beta cells, and type 2 diabetes (T2D) occurs from insulin resistance and relative insulin deficiency (Chaudhury *et al.*, 2017; Iwasaki *et al.*, 2023). As a consequence, blood glucose levels remain elevated because target tissues are unable to respond adequately to circulating insulin, thereby increasing glucotoxicity (Matafome *et al.*, 2013). Glucotoxicity is a condition of chronic high glucose levels, possessing several detrimental effects on cells and tissues. It is closely associated with the progressive dysfunction and apoptosis of pancreatic β-cells, which ultimately contribute to insulin resistance and exacerbate hyperglycemia. The mechanisms underlying glucotoxicity involve the

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generation of reactive oxygen species (ROS), the formation of advanced glycation end products (AGEs), and further activation of pathways such as RAGE signaling and pro-inflammatory cytokines. These factors collectively create an environment of oxidative stress, inflammation, and cellular damage, playing pivotal roles in the development of diabetes-related complications (Van Eupen *et al.*, 2013; Vlassara and Uribarri, 2014). Among several critical factors contributing to the glucotoxicity related to diabetes is the formation and accumulation of AGEs. AGEs formation is facilitated by various reactive di-carbonyl compounds, commonly the by-products of glucose metabolism, namely glucose, GO, and methyl glyoxal (MGO) etc. Interestingly, this critical condition of glucotoxicity requires specific attention to mitigate the adverse effects of AGEs formation and accumulation. However, the current treatment and management of glucotoxicity is focused on controlling the blood glucose levels. These current treatments for glucotoxicity are the same as T2DM, which include insulin therapy and oral medications like metformin and thiazolidinediones (Khan M. S., 2012), having many limitations, such as side effects, resistance over time, and challenges with patient adherence etc. (Chaudhury *et al.*, 2017) (Ocvirk *et al.*, 2013). These limitations in controlling glucotoxicity have driven interest in discovering new natural compounds that might offer anti-glucotoxic benefits with fewer adverse effects and better patient compliance.

Since ancient times, plants have been used as a source of medicine for the identification, management, and eradication of any medical, psychological, or social imbalance. Medicinal plants have been utilized for centuries as a cornerstone of traditional medicine (Boy *et al.*, 2018) and are increasingly recognized for their therapeutic potential such as antimicrobial, antiulcer, anti-inflammatory, anti-diabetic, and antioxidant properties in modern pharmacology (Rahman *et al.*, 2021; Sharma and Tripathi, 2021). Different parts of these traditional medicinal plant such as leaves, root, fruits, barks, seed, stem, rhizome etc., containing essential phytochemicals, have been used traditionally for the new drug discovery of synthetic and semisynthetic pharmaceuticals (Biswas and Mukherjee, 2003). Among several medicinal plants such as, *Curcuma longa (C. longa), Aloe vera*, *Azadirachta indica* (Neem) etc. has been widely used traditionally due to their well-known properties such as anti-inflammatory, anti-diabetic, wound-healing properties, antimicrobial properties etc. (Sharma and Tripathi 2021; Z *et al.*, 2019). In particular, *C. longa*, has various significant pharmacological effects, including anti-inflammatory, anticancer, antimicrobial and antioxidant properties (Jaradat *et al.*, 2017; Zari and Zari 2015). *C. longa*, a plant widely used in traditional medicine and culinary practices, especially in south asian countries. *C. longa* contains diverse bioactive ingredients including curcuminoids (Curcumin, desmethoxycurcumin, bisdemethoxycurcumin) and essential oils found primarily in the rhizomes (Aggarwal *et al.*, 2013). Modern research has increasingly validated its potential health benefits, attributing many of its effects due to the presence of primary active compound, curcumin. Curcumin of *C. longa* , has gained significant attention for its potential antidiabetic properties, anti-inflammatory, antioxidant, and insulin-sensitizing effects (Khan *et al.*, 2012). Several *in vivo* studies have shown that curcumin shows hypoglycemic and hypolipidemic effects in diabetic animal models (Khattak *et al.*, 2005). However, there is very few studies, which have highlighted the inhibitory effect of curcumin on AGE formation, and most of them are based on molecular docking studies. Despite promising findings, the molecular mechanisms of curcumin's interaction with RAGE and its combined anti-glycation and metabolic effects in T2DM remain unclear. Although, the antidiabetic effect of curcumin has been reported both *in vivo* and *in vitro* through different pathways, but there are very few studies has been focused on glucotoxicity. Moreover, in Bangladesh, there is scarcity in research, based on curcumin against AGEs inhibition. This research gap led to the performance of this study, which focused on investigating the molecular mechanism of curcumin in diabetes, focusing on glucotoxicity.

Therefore, this study aims to reveal the potential of curcumin as a novel antidiabetic agent in the AGEs-induced diabetic model as a natural, cost-effective traditional diabetic therapy, especially for the management of highly rising complications associated with diabetes.

Materials and Methods

Chemicals and reagents: Bovine serum albumin (BSA), Glyoxal (GO, 40% solution in H_2O), Phosphate-buffered saline (PBS, pH 7.4). Sodium azide was acquired through commercial sources. The additional chemical reagents, including FeCl3, Wegener's reagent, Fehling's Solution, chloroform, H_2SO_4, HCl, alkaline reagent, ethanol, n-hexane, and ethyl acetate, were obtained from the laboratory at Jagannath University. Metformin was a generous gift of Incepta Pharmaceuticals Ltd. For the *in vivo* experiment. Around forty (40) Swiss albino mice were acquired from Jahangirnagar University and subsequently bred in the laboratory of the Pharmacy Department at Jahangirnagar University.

Collection of plant material and identification: The rhizomes of fresh turmeric were collected from Jamalpur Sadar, Mymensingh, Bangladesh, during the month of March 2024. The authentication of the plant sample was confirmed by the National Herbarium's taxonomist (Accession Number-103370), and a voucher specimen of this plant was deposited at the Bangladesh National Herbarium, Dhaka, Bangladesh.

Extract preparation: The collected rhizomes of *C. longa* were cleaned to eliminate the unwanted materials and then subjected to sun drying for two weeks, and then the fine powdered sample was obtained from these dried rhizomes through grinding (Nagmoti *et al.*, 2015). Afterwards, 50g of powdered materials were soaked in 500 ml of ethanol for 10 days. Then, the mixture was kept in a cool and clean place covered with aluminum foil paper for 10 days with occasional vigorous shaking and stirring. Afterwards, the mixture was filtered using a vacuum filtration process. Upon filtration, 400 ml of filtrate was subjected to reflux condensation to remove ethanol from the extract, and the remaining solvent was then evaporated using a water bath. The resultant concentrated extract (around 6.4 g) had a yield value of 12.8 ± 0.5 %, and the CLE was stored in freezing conditions (4°C) for further analysis.

Phytochemical screening: Phytochemical screening involves qualitative detection of bioactive compounds in CLE, revealing its therapeutic properties through the presence of alkaloids, flavonoids, tannins, saponins, phenols, and glycosides, etc. The tests are accomplished by following the methods used in the previous studies (Cruz *et al.*, 2022; Parveen *et al.*, 2018; Wang *et al.*, 2019). Briefly, Wagner's test was performed by adding dilute HCl to the plant extract, resulting in a reddish-brown precipitate indicating the presence of alkaloids. Alkaline reagent tests were conducted for detecting flavonoids by noting a brilliant yellow tint that becomes colorless when diluted HCl is added. Phenolic compounds were detected using a ferric chloride test, which resulted in the formation of a greenish-black color. Tannins were detected through the ferric chloride test by forming a dark green color when a mixture of plant extract and 5% ferric chloride solution was mixed. Salkowski's test was used to detect the terpenoids by mixing 0.5 ml of crude extract with 2 ml of chloroform and 3 ml of H_2SO_4. The presence of coumarins was confirmed by adding 1.5 ml of 10% NaOH to 2 ml of the plant extract, which resulted in a bright yellow color. Salkowski's test confirmed the presence of steroids by mixing crude extract with chloroform and H_2SO_4, with the appearance of a red interface.

Determination of the % curcumin percentage and color value: The curcumin percentage and color value were determined via UV/Visible spectrophotometry, following a previously described method (Ahmed *et al.*, 2020; Zhang and Li 2018). For the determination of curcumin, 0.1 g dried CLE was dissolved in 25 ml of ethanol, filtered, and reconstituted to 100 ml. The absorbance was measured at 425 nm using a UV machine. The percentage of curcumin in the CLE was calculated by using the following formula:

% Curcumin = ax100/(L x A x W)

Where, a = absorbance of the sample at 425 nm; L = path length (1 cm); A = absorptivity of curcumin from referenced standard; W = weight of the extract in grams.

The color value was determined using the formula as followings:

Color value = a×1000

Biological investigations: In vitro assays

Cytotoxicity assay: Cytotoxicity assay was performed through brine shrimp lethality assay, involving the use of control groups to ensure the results are solely attributed to the test agent's activity (Hou *et al.,* 2016; Jahan *et al.,* 2011; Md Sumsuzzman *et al.,* 2016). Therefore, vincristine sulphate, was used as a test agent to consider as positive control group. Briefly, vincristine sulphate was dissolved in DMSO initially to create various concentrations, which were further added to simulated seawater to create the positive control groups. Finally, the obtained results of test agents were compared with the positive control group, ensuring a comprehensive understanding of the cytotoxic effects. Three glass vials containing 5 ml of simulated seawater and 10 shrimp nauplii were used as control groups. If brine shrimps showed a rapid mortality rate, the test was considered invalid, as the nauplii died due to other reasons. After 24 hours, vials were inspected, and survivors were counted. Percent mortality was calculated for each dilution. Concentration-mortality data were analyzed using linear regression, with the median lethal concentration (LC_{50}) representing the chemical's effectiveness.

Anti-glycation assay: Antiglycation efficacy of CLE was evaluated through the GO-AGEs assay. GO-derived AGEs were prepared by following a previously described method (Chen *et al.,* 2022; Spagnuolo et al. 2021). Firstly, 20 mM glyoxal solution was prepared by diluting 40% glyoxal solution in PBS. Then, individual mixtures containing BSA (10 mg/ml), glyoxal (20 mM), turmeric extract, or metformin were prepared separately. Then, sodium azide (0.02% w/v) was added to prevent microbial contamination. Afterwards, the mixtures were incubated at 37°C for 7 days under sterile conditions to facilitate AGE formation. Metformin (25 mg/ml stock solution) was used as the positive control. Then, 100 µL from each reaction mixture was transferred into a 96-well microplate in triplicate. The absorbance was measured at 450 nm using a microplate reader. Finally, the mean OD values for each group were calculated and the percentage inhibition of glycation was determined by using the following formula:

% Inhibition of AGE formation = (1-OD value of the sample/ OD value of the control) X 100

Antioxidant activity: The free radical scavenging activities (antioxidant capacity) of CLE were estimated by the DPPH (2,2-Diphenyl-1-picrylhydrazyl) free radical scavenging assay following the previous method (Ali *et al.,* 2021; Hakiman 2012). DPPH radical scavenging activity is described as IC50, which is the concentration of samples to produce 50% reduction of the DPPH(Kang *et al.,* 2014; Wu *et al.,* 2020). Calculated amount of ASA was dissolved in methanol to get a mother solution having a concentration of 1000 µg/ml. Then the mother solution was serially diluted to get different concentrations from 500.0 to 0.977 µg/ml. The calculated amount of turmeric extract was measured and dissolved in methanol to get a mother solution having a concentration of 1000 µg/ml. After that 20 mg of DPPH was weighed and dissolved in 1 liter of methanol to get a DPPH solution having a concentration of 20 µg/ml, as well as 2.0 ml of a methanol solution of the sample (Control/extractives) at different concentrations (500.0 to 0.977µg/ml) were mixed with 3.0 ml of a DPPH methanol solution (20 µg/ml). After 30 minutes, the mixtures were kept at room temperature in a dark place, and the absorbance was measured at 517 nm against methanol as a blank by a UV spectrophotometer. The inhibition of free radical DPPH in percent (I %) was calculated as follows:

$(I\ \%) = (1-A_{sample}/A_{blank})\ X\ 100$

Where, A_{blank} = Absorbance of control reaction (containing all reagents except the crude turmeric extract) and A_{sample} = Absorbance of CLE. The percentage of inhibition (I %), was determined by plotting the inhibition percentage against the extract concentration.

Biological investigations: In vivo assays

Antidiabetic activity:

Experimental animals: Swiss albino mice (male and female) having an average weight of 20-25 g were used in this study. All mice were collected from the Animal Resources Branch of Jahangirnagar University. Animals were preserved at the laboratory of standard environment of 25°C temperature, 55-60% relative humidity on 12h light/dark cycle. Adequately formulated food and water supply were also maintained.

Ethical statement: The experimental procedure involving mice in this study was conducted in strict accordance with the ethical guidelines for the care and use of laboratory animals. Ethical approval for the research was obtained from the Ethical Review Committee of Jagannath University, Dhaka, Bangladesh.

Experimental designs for antidiabetic activity: The anti-diabetic activity of CLE was investigated in this glyoxal-induced diabetic mouse model following previous studies *(Chen et al.,* 2021; Zhang *et al.*, 2019). Briefly, diabetes was induced in experimental mice using glyoxal and glucose for their established roles in exacerbating glucotoxicity, a key contributor to the development of diabetes and related complications. Glyoxal solution (40% in water) was procured from Sigma-Aldrich. Stock solutions were prepared by diluting the compounds with sterile distilled water to the desired concentration (1%) for administration. Additionally, 10% glucose solution was prepared by dissolving 10 gm glucose in 100 ml of distilled water, ensuring a dosage of glucose 2 gm/kg of body weight. The study examined the hypoglycemic effect of CLE at 200 and 400 mg/kg doses, comparing results to control and standard groups with metformin as the standard drug. For conducting the anti-diabetic study, the five different groups, namely negative control, diabetic control, positive control, CLE group at low (200 mg/kg of body weight) and high dose (400 mg/kg of body weight), containing three mice in each group, were included. The animals were identified by their tail numbers, as it was difficult to observe the biological response of three mice receiving the same supplement simultaneously. Each group underwent the specific treatment course for 21 days, with the average weight determined before and after completion of the experiment. This study involved preparing suspensions of test samples for different doses of crude turmeric extract. The 200 mg/kg dose was prepared by measuring 200 mg of extract and triturating it with Tween-80. The 400 mg/kg dose was prepared by dissolving 200 mg of extract in distilled water. The standard metformin dose was prepared by triturating 250 mg of extract with tween-80 and adjusting with distilled water, and the control solution was prepared by mixing tween-80 with normal saline.

Measurement of blood glucose levels: Blood glucose levels were measured at Day 1, Day 7, Day 14, and Day 21 through the Best-Check Basic glucometer by using test strips to assess the anti-diabetic efficacy of CLE in different treatment groups.

Dissection of mice for visual examination of internal organs: Following the completion of the anti-diabetic study on mice, four mice were randomly selected, one from each experimental group, for dissection and visual examination of internal organs. The groups included the diabetic control group, the standard treatment group (metformin), and two groups treated with CLE at two different doses, 200 mg/kg and 400 mg/kg body weight.

Molecular docking study: Molecular docking study predicts the interaction between small molecules and target proteins, therefore offering insights into binding affinity and possible biological activity (Sindhuja *et al.,* 2021). Molecular docking was employed in this work to investigate the binding interactions of curcumin, a bioactive component of CLE, with the Receptor for Advanced glycation end

products (RAGE), a key player in glucotoxicity and diabetes-related complications. These pathways included interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), protein kinase B (Akt1), nuclear factor kappa B (NF-κB), Janus kinase (JAK), signal transducer and activator of transcription (STAT), c-Jun N-terminal kinase (JNK), Ras, mammalian target of rapamycin (mTOR), and cyclin-dependent kinase inhibitor 1 (P21). The methodologies for performing the molecular docking studies were carried out following previous studies (Kwon *et al.*,2022; Razia *et al.*, 2021).

Results and Discussion

Glucotoxicity describes the harmful impact of persistently elevated glucose levels in the blood and is mainly linked to type 2 diabetes mellitus. This condition causes persistent hyperglycemia, which ultimately results in pancreatic β-cell dysfunction, diminished insulin secretion, and increased insulin resistance. Further, persistent hyperglycemia overstimulates pancreatic β-cells, ultimately promoting abnormalities and apoptosis by causing uncontrolled oxidative stress as well as protein glycation (*MC et al., 2005; Morioka et al., 2017; Vistoli et al.,* 2013). Based on previous studies, traditional medicinal plants, containing numerous natural bioactive compounds, are crucial for the healing of diabetes related complexities, including glucotoxicity (Parveen *et al.*, 2018; Yadav *et al.*, 2023). Among different bioactive compounds of traditional medicinal plants, curcumin enhances insulin sensitivity by modulating key signaling pathways, including the RAGE signaling pathway as well as the up-regulation of insulin-like growth factor (IGF)-1R and PI3K, crucial for glucose metabolism (Alam *et al.*, 2022; Parveen *et al.*, 2021). Therefore, this study has been designed to evaluate the pharmacological potential of *C. Longa* in glucotoxicity through in vitro, in vivo, and molecular docking analyses, with a focus on its efficacy against glucotoxicity by targeting the AGE signaling pathway.

Phytochemical screening results: The phytochemical analysis of *C. longa* in different solvents (n-Hexane, chloroform, ethyl Acetate, aqueous, and ethanol) revealed the presence of various bioactive compounds. The confirmation of their presence is represented as "+" for low, "++" for moderate, "+++" for high, and "-" for absence. The findings are summarized in table 1.

Table 1. Phytochemical screening test results in different fractions of *C. longa* are given bellow.

Phytochemicals	Test	n-Hexane	Chloroform	Ethyl acetate	Aqueous	Ethanol
Alkaloids	Wagner's test	+++	-	++	-	+++
Flavonoids	Alkaline reagent test	+	-	++	+++	+++
Phenol	Ferric chloride test/ Alkaline test	-	-	++	+++	+++
Tannins	Ferric chloride test/ Alkaline test	-	-	++	+++	+++
Terpenoids	Salkowski test	-	+++	+	-	+++
Saponins	Frothing test	-	+++	-	-	+++
Coumarin	Alkaline reagent test	-	-	+++	++	+++
Steroids	Salkowski test	-	+++	+	-	+++

Cytotoxicity assay results: The lethal concentration (LC_{50}) of the test samples after 24 hrs was obtained by plotting of the percentage of the shrimps that died against the logarithm of the sample concentration, and the best-fit line was obtained from the curve data by means of regression analysis (Figure 1A-B). Vincristine sulfate (VS) was used as positive control for which the LC_{50} was found to be 0.464 µg/ml. In a comparison with the negative control, VS (positive control) gave significant mortality, and the LC_{50} value of 3.94 µg/ml of the extract was compared to this positive control. However, varying degree of lethality to artemia salina was observed with exposure to different dose levels of the test samples. The degree of lethality was directly proportional to the gradual increase of

concentration of the extract, ranging from the lowest concentration (0.78125 µg/ml) to the highest concentration (400 µg/ml). Maximum mortalities took place at a concentration of 400 µg/ml, whereas the least mortalities were at 0.78125 µg/ml concentration. In other words, mortality increased gradually with the increase in concentration of the test samples.

Antioxidant activity: From the obtained results of antioxidant activity tests, the crude ethanolic extract showed significant free radical scavenging activity with an IC50 value of 28.20 µg/ml, compared to the standard ascorbic acid of 31.25 µg/ml, as shown in Figure 2(A-B).

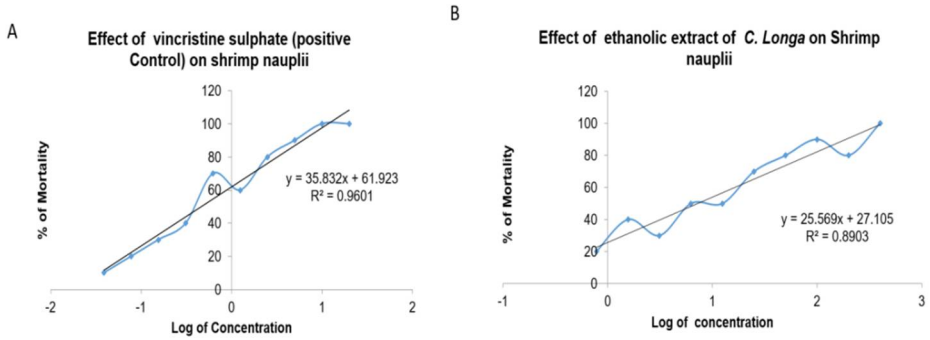

Figure 1. Cytotoxicity effect of CLE. A) Plot of % mortality and predicted regression line of Vincristine sulphate. B) Plot of % mortality and predicted regression line of CLE.

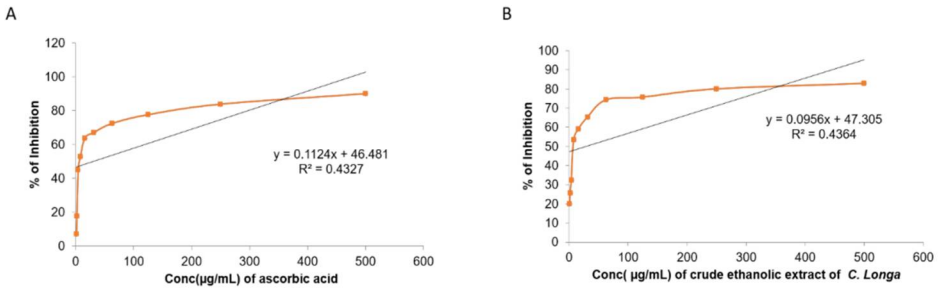

Figure 2. Antioxidant potential of CLE. A) Plot of % inhibition and predicted regression line of ascorbic acid B) Plot of % inhibition and predicted regression line of CLE.

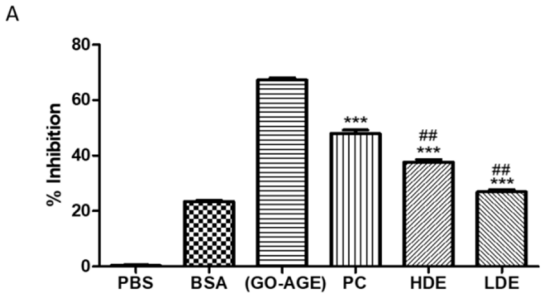

Figure 3. Anti-glycation potential of CLE. A) Inhibitory percentages were plotted against different groups, including PBS= Phosphate buffer Saline, BSA= Bovine Serum Albumin, GO-AGE= Glyoxal derived advanced glycation end products, HDE= High dose of CLE; LDE= Low dose of CLE. Each value represents the mean ± SEM of triplicate experiments. Here, data are considered statistically significant, where (***) $p < 0.001$ compared to control group (GO-AGE's group) and (##) $p < 0.01$, compared to positive control group.

Anti-glycation activity: The antiglycation activity of CLE was evaluated by calculating the percentage of inhibition of GO-AGEs formation among different groups, including PBS (control), BSA (blank), metformin (standard), and two different doses of CLE(200 mg and 400 mg). The results, as illustrated in the Figure 3-A, showed that the BSA group showed minimal inhibition at around 22.8%, the standard group (metformin) demonstrated significant anti-glycation activity with 48.2% inhibition. Among the crude turmeric extract-treated groups, the high dose (400 mg/kg) exhibited greater inhibition (39.3%) than the low dose (28.5%), indicating a dose-dependent antiglycation effect.

***Anti-diabetic activity study*:**

Effect of CLE on blood glucose level

Effect of CLE on the average weight: The changes in average body weight among different experimental groups of mice over 21-day study periods are summarized in Figure 4**A**. On Day 0, there were no significant differences in the average weights across all groups. By Day 7, the diabetic control group showed an increase in body weight compared to the normal control group, indicating hyperglycemia-induced weight gain. This trend continued until Day 21, when the diabetic control group was recorded with the highest body weight (30.3 g), compared to the normal control group (25.8g). In contrast, the groups treated with CLE at low (200 mg/kg) and high doses (400 mg/kg) exhibited a moderated weight gain compared to the diabetic control group. By Day 21, the high-dose group maintained an average weight of 27.9 g, while the low-dose group showed a similar trend with 28.0 g. This suggests a dose-dependent normalization of weight in CLE groups. The metformin-treated group (positive control) also exhibited controlled weight gain, with an average weight of 27.7 ± 0.330 g on Day 21, highlighting the therapeutic potential of CLE.

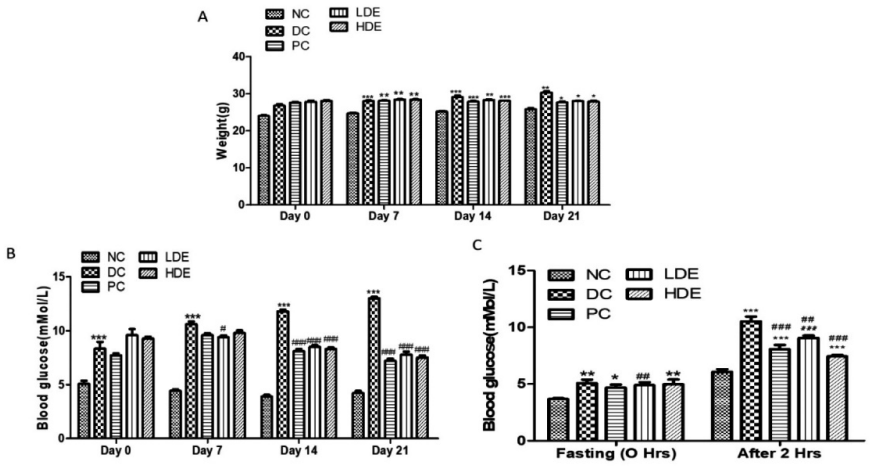

Figure 4. Antidiabetic effect of CLE in diabetic mice model. A) Effect of CLE on the average weight, B) Effect of CLE on Blood Glucose Level in diabetic mice model, C) Effect of CLE on Oral Glucose Tolerance Test (OGTT). Each value represents the mean ± SEM of triplicate experiments. Here, data are considered statistically significant, where (***) p <0.001, (**) p <0.01, and (*) p <0.1 compared to the control group (GO-AGE's group) whereas (###) p <0.001, (##) p <0.01, and (#) p <0.1 compared to positive control group

Effect of CLE on oral glucose tolerance test (OGTT): OGTT was conducted on the 7th day to assess the ability of the test groups to regulate blood glucose levels. The results are presented in Figure **4C**. In the normal control group, the fasting blood glucose level was observed as 3.8 mmol/l, which increased to 4.4 mmol/l after 2 hours of glucose intake, indicating normal glucose tolerance. In the diabetic control group, fasting blood glucose level was 6.4 mmol/l, which significantly increased to 10.6

mmol/L after glucose intake, confirming the impaired glucose tolerance. The positive control (metformin) group indicated significant glucose reduction (8.7 mmol/l) compared to the diabetic control group. Interestingly, CLE at both doses was able to reduce blood glucose levels in the OGTT in a dose-dependent manner. Shortly, the low dose (200 mg) of CLE demonstrated very moderate glucose tolerance, with the range of blood glucose levels to 9.1 mmol/l after 2 hours of glucose intake, in comparison to the diabetic control group. However, the high dose (400 mg) of CLE has shown significant reduction in glucose regulation compared to the diabetic control group.

Effect of CLE on blood glucose level in diabetic mice model: The effects of CLE on blood glucose levels were evaluated over 21 days. The findings are presented in Figure 4B. Blood glucose levels remained consistent over the experimental period, with 4.2 mmol/L recorded on day 21 whereas levels peaked at 10.6 mmol/L of diabetic control group by day 21, reflecting sustained hyperglycemia. In positive control (metformin) group, blood glucose levels reduced significantly to 7.2 mmol/l on day 21. The low dose (200 mg) of CLE suggested moderate anti-diabetic activity whereas high dose (400 mg) indicated significant activity on a dose dependent manner.

Visual observations of internal organs: For the visual observation of the anti-diabetic effect of CLE, dissection of different organs was performed under sterile conditions, and the internal organs, including the liver, kidney, and heart, were carefully examined for any visible abnormalities, discoloration, or structural defects. This step was performed to assess the potential toxicity or adverse effects of CLE on vital organs. However, no significant abnormalities were observed, suggesting that the turmeric extract was well tolerated at the administered doses. This aligns with previous findings on the treatment of CLE at safety doses in animal models (Figure 5A-C). However, in-depth analysis of organ toxicity, including histopathology, various liver enzyme tests (ALT, AST) and other kidney markers (Creatinine, urea) etc. will be conducted in further study.

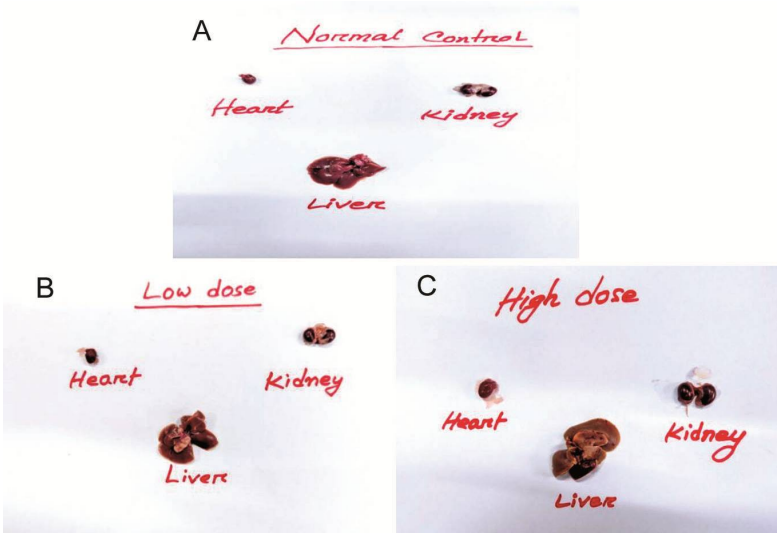

Figure 5. Visual observation of vital organs of mice. A) Effect of CLE on the normal control group B) Effect of CLE at low dose of CLE, and C) Effect of CLE at high dose

Statistical evaluation of the anti-diabetic study data: All data were expressed as percentage and mean ± Standard Error Mean (SEM). Statistical significance between groups were determined by using two-way ANOVA and Bonferroni posttests were performed between multiple groups. One-way

ANOVA and using Student's t- test unpaired between two groups. P<0.05 were considered statistically significant.

Determination of % curcumin and color value: The percentage of curcumin in the turmeric extract was 21.07%, indicating a high concentration of the bioactive compound in the sample. Besides that, the strong color intensity further correlate the high curcumin content, as curcumin is the primary compound responsible for turmeric's vibrant yellow color.

Molecular docking study results: Initially, the comparative docking analysis of curcumin, desmethoxycurcumin, and bisdemethoxycurcumin was conducted with the RAGE protein (PDB ID: 4YBH) presented in Table 2. Among these, curcumin demonstrated the highest binding affinity, suggesting its superior potentiality in modulating RAGE-mediated pathways. Based on this finding, further molecular docking studies were carried out to explore the interaction of curcumin with other proteins involved in the downstream signaling pathways of RAGE as shown in Figure 6 and 7. From the obtained results, curcumin demonstrated significant binding affinity with all the proteins, indicating strong interactions that support its therapeutic potential. As CLE contains many other compounds than curcumin, therefore synergistic effect can be responsible for its claimed anti-diabetic effect. However, further studies (preclinical, clinical) required, apart from this primary evidence to establish curcumin and *C. longa* as new candidate.

Table 2. Interaction comparison among curcumin against different proteins of the RAGE signaling pathway related proteins.

Protein (PDB ID)	Compounds	Binding Affinity (kcal/mol)
	Curcumin	-6.6
4YBH (RAGE)	Desmethoxycurcumin	-6.5
	Bisdemethoxycurcumin	-6.3
1ALU (IL-6)		-6.0
1A8M (TNF-α)		-6.3
1HIB (Akt1)		-7.1
1UNP (NF-κB)		-6.2
3EYG (JAK)	Curcumin	-7.6
3T92 (CHOP)		-6.9
4H39 (JNK)		-6.5
3WWT (STAT)		-6.0
4L8G (RAS)		-8.3
5WBH (mTOR)		-7.6
821P (P21)		-9.1

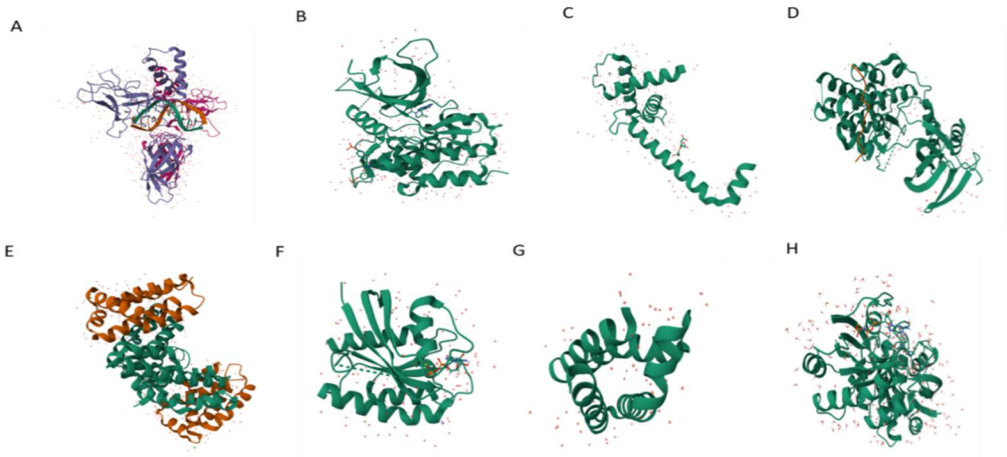

Figure 6. Molecular docking study results (3D view) of curcumin and different proteins of the RAGE signaling pathway proteins (A) 1A3Q, (B) 3EYG, (C) 3T92, (D) 4H39, (E) 3WWT, (F) 4L8G, (G) 5WBH, (H) 821P.

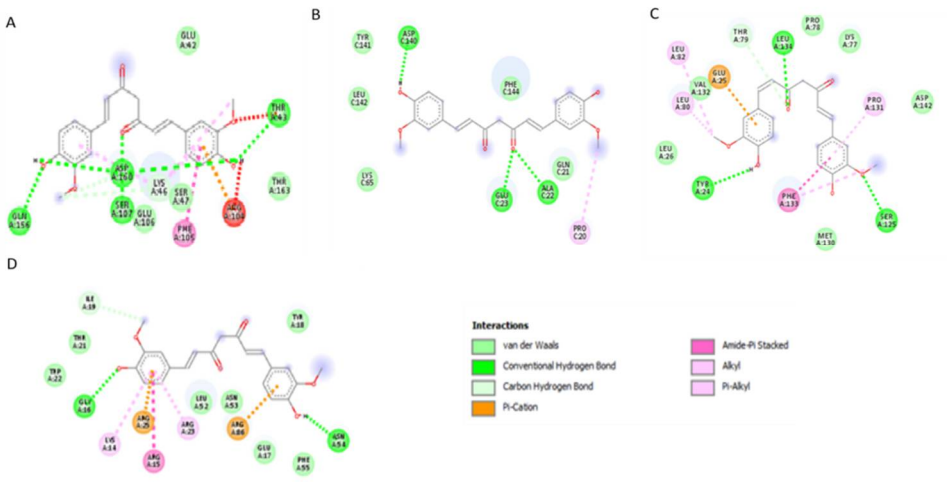

Figure 7. Molecular docking study results (2D view) of curcumin and different proteins of the RAGE signaling pathway proteins (A) 1A3Q, (B) 3EYG, (C) 3T92, (D) 4H39.

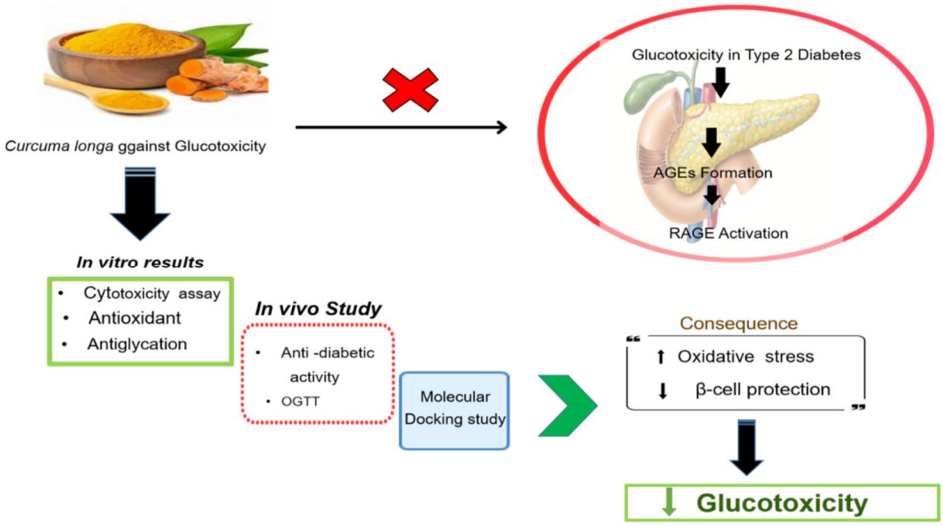

Figure 8. Possible mechanism of *Curcuma longa* against glucotoxicity.

Conclusion

This study is comprehensively designed to explore the pharmacological potential of *C. longa* through *in vitro*, *in vivo*, and molecular docking analyses, with a focus on its efficacy against glucotoxicity and diabetes-related complications. The brine shrimp cytotoxicity assays demonstrated toxicity at higher doses, and indicate its safety at lower concentrations for therapeutic use. However, in vitro experiments included the anti-glycation and antioxidant activities of CLE, indicating its inhibitory capacity against protein glycation and neutralizing the free radicals effectively. *In vivo* studies also highlighted the anti-diabetic activity of CLE, reflected by improved glucose regulation in both high and low doses at almost the same degree. Besides that, the quantitative determination of curcumin in CLE confirms its presence in the highest quantity. Furthermore, molecular docking study results provide preliminary evidence that curcumin has proven its potential as a strong candidate, specifically through its interaction with the RAGE and its associated signaling pathways. Overall, the results of this research support the traditional use of CLE as a natural agent in managing diabetes and its complications while also underscoring the need for

further studies. Since the study couldn't differentiate between the effects of low and high doses, dose optimization becomes essential to identify the most effective therapeutic concentration. Repeating this study in the same model with a refined dose range or conducting it in a different model could provide better insights into the dose-response relationship and enhance the reliability of the findings.

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Conflict of interest

The authors declare that they have no conflicts of interest.

References

Aggarwal, B.B., Gupta, S.C. and Sung, B. 2013. Curcumin: An orally bioavailable blocker of TNF and other pro-inflammatory biomarkers. *Br. J. Pharmacol.* **169**, 1672-1692.

Ahmed, O.A.A., El-Bassossy, H.M., Azhar, A.S., Tarkhan, M.M. and El-Missiry, M.M. 2020. Interference with AGEs formation and AGEs-induced vascular injury mediates curcumin vascular protection in metabolic syndrome. *Sci. Rep.* **10**, 315

Alam, S., Sarker, M.M.R., Sultana, T.N., Chowdhury, M.N.R., Rashid, M.A., Chaity, N.I. and Mohamed, I.N. 2022. Antidiabetic phytochemicals from medicinal plants: Prospective candidates for new drug discovery and development. *Front. Endocrinol.* **13**, 800714.

Ali, M.S., Sayem, S.A.J., Habibullah, Quah, Y., Lee, E.-B., Birhanu, B.T. and Park, S.-C. 2021. Investigation of potential antioxidants, thrombolytic and neuropharmacological activities of Homalomena aromatica leaves using experimental and in silico approaches. *Molecules*. **26**, 975.

Biswas, T.K. and Mukherjee, B. 2003. Plant medicines of Indian origin for wound healing activity: A review. Int. J. Low. *Extrem. Wounds.* **2**, 25-39.

Boy, H.I.A., Rutilla, A.J.H., Santos, K.A., Ty, A.M.T., Yu, A.I., Mahboob, T. and Nissapatorn, V. 2018. Recommended medicinal plants as source of natural products: A review. *Digit. Chin. Med.* **1**, 131-142.

Chaudhury, A., Duvoor, C., Reddy Dendi, V.S., Kraleti, S., Chada, A., Ravilla, R. and Mirza, W. 2017. Clinical review of antidiabetic drugs: Implications for type 2 diabetes mellitus management. *Front. Endocrinol.* **8**, 6.

Chen, C.Y., Zhang, J.Q., Li, L., Guo, M.M., He, Y.F., Dong, Y.M. and Yi, F. 2022. Advanced glycation end products in the skin: Molecular mechanisms, methods of measurement, and inhibitory pathways. *Front. Med.* **9**, 1-17.

Chen, X., Yu, M., Xu, W., Zou, L., Ye, J., Liu, Y., Xiao, Y. and Luo, J., 2021. Rutin inhibited the advanced glycation end products-stimulated inflammatory response and extra-cellular matrix degeneration via targeting TRAF-6 and BCL-2 proteins in mouse model of osteoarthritis. *Aging (Albany. NY).* **13**, 22134-22147.

Cruz, J.M. dos A., Corrêa, R.F., Lamarão, C.V., Sanches, E.A., Campelo, P.H. and Bezerra, J. de A. 2022. Ficus spp.: Phytochemical composition and medicinal potential. *Res. Soc. Dev.* **11**, 12.

Ghani, A. 2003. Medicinal plants of Bangladesh with chemical constituents and uses. *Asiatic Soc. Bangladesh.*1-16.

Khan, H.M.S. 2012. Evidence based study of side effects of drugs used in the treatment of diabetes mellitus. *Afr. J. Microbiol. Res.* **6**, 1805-1808.

Hakiman, M. 2012. Total antioxidant, polyphenol, phenolic acid, and flavonoid content in *Ficus deltoidea* varieties. *J. Med. Plants Res.* **6**, 4776-4784.

Hou, Q., Li, M., Lu, Y.H., Liu, D.H. and Li, C.C. 2016. Burn wound healing properties of asiaticoside and madecassoside. *Exp. Ther. Med.* **12**, 1269.

Iwasaki, K., Abarca, C. and Aguayo-Mazzucato, C. 2023. Regulation of cellular senescence in type 2 diabetes mellitus: From mechanisms to clinical applications. *Diabetes Metab. J.* **47**, 441-453.

Jahan, T., Shahreen, S., Banik, J., Islam, F., Al-Mamun, A., Das, R., Rahman, S., Seraj, S., Jahan, R., Rahmatullah, M., 2011. Antinociceptive activity studies with methanol extracts of Ficus Hispida L.f. leaves and fruits in Swiss albino mice. *Adv. Nat. Appl. Sci.* **5**, 131-135.

Jaradat, N. A., Zaid, A. N., Al-Ramahi, R., Alqub, M. A., Hussein, F., Hamdan, Z. and Ali, I. 2017. Ethnopharmacological survey of medicinal plants for urological diseases in Palestine. *BMC Complement. Altern. Med.* **17**, 1-18.

Kang, M.C., Kim, S.Y., Kim, Y.T., Kim, E.A., Lee, S.H., Ko, S.C. and Jeon, Y.J. 2014. *In vitro* and *in vivo* antioxidant activities of polysaccharides from *Aloe vera* gel. *Carbohydr. Polym.* **99**, 365-371.

Khan, N., Shreaz, S., Bhatia, R., Ahmad, S.I., Muralidhar, S., Manzoor, N. and Khan, L.A. 2012. Anticandidal activity of curcumin and methyl cinnamaldehyde. *Fitoterapia* . **83**, 434-440.

Khattak, S., Saeed-ur-Rehman, Shah, H. U., Ahmad, W. and Ahmad, M. 2005. Biological effects of Curcuma longa and Alpinia galanga. *Fitoterapia*.**76**, 254-257.

Kwon, R.-H., Thaku, N., Timalsina, B., Park, S.-E., Choi, J.-S. and Jung, H.-A. 2022. Inhibition mechanism of Morus alba components on diabetes via docking analyses. *Antioxidants*. **11**, 383.

Maessen, D.E.M., Stehouwer, C.D.A. and Schalkwijk, C.G. 2015. The role of methylglyoxal in diabetes and age-related diseases. *Clin. Sci.* **128**, 839-861.

Matafome, P., Sena, C. and Seiça, R. 2013. Methylglyoxal, obesity, and diabetes. *Endocrine*. **43**, 472-484.

MC, T., JW, B., SR, T. and ME, C. 2005. The role of AGEs in diabetic cardiovascular disease. *Curr. Drug Targets*. **6**, 453-474.

Md Sumsuzzman, D., Chowdhury, T.F., Bulbul, I.J., Begum, Y. and Professor, A. 2016. Antibacterial, antioxidant, and cytotoxic activities of *Ficus hispida*. *Int. J. Adv. Pharm. Sci.* **3**, 841-845.

Morioka, Y., Teshigawara, K., Tomono, Y., Wang, D., Izushi, Y., Wake, H. and Nishibori, M. 2017. Localization of AGEs in rat pancreatic islets. *J. Pharmacol. Sci.* **134**, 218-224.

Nagmoti, D.M., Kothavade, P.S., Bulani, V.D., Gawali, N.B. and Juvekar, A.R. 2015. Antidiabetic activity of Pithecellobium dulce seeds. *Eur. J. Integr. Med.* **7**, 263-273.

Ocvirk, S., Kistler, M., Khan, S., Talukder, S.H. and Hauner, H. 2013.Traditional medicinal plants used for the treatment of diabetes in rural and urban areas of Dhaka, Bangladesh--an ethnobotanical survey. *J. Ethnobiol. Ethnomed.* **9**, 43.

Parveen, A., Kim, J.H., Oh, B.G., Subedi, L., Khan, Z. and Kim, S.Y. 2018. Phytochemicals: Target-based therapeutic strategy for diabetic retinopathy. *Molecules.* **23**(7), 1519.

Parveen, A., Sultana, R., Lee, S.M., Kim, T.H. and Kim, S.Y. 2021. Phytochemicals against diabetic complications targeting AGEs. *Arch. Pharm. Res.* **44**, 378-401.

Rahman, M.M., Uddin, M.J., Ali Reza, A.S.M., Tareq, A.M., Emran, T.B. and Simal-Gandara, J. 2021. Ethnomedicinal value of antidiabetic plants in Bangladesh. *Plants*. **10**, 1-31.

Sharma, R. and Tripathi, A. 2021. Indigenous medicinal plants and applications. *J. Pharm. Res. Int.* **33**, 160-166.

Sindhuja, A., Vimalavathini, R. R. and Kavimani, S. 2021. In silico antiglycation activity of isorhamnetin. *Biomed. Pharmacol. J.* **14**, 2299-2306.

Spagnuolo, L., Posta, S. D., Fanali, C., Dugo, L. and De Gara, L. 2021. Antioxidant and antiglycation effects of polyphenols. *Antioxidants.* **10**, 424.

Talukder, A. and Hossain, M. Z. 2020. Prevalence of diabetes mellitus in Bangladesh. *Sci. Rep.* **10**, 10237.

Van Eupen, M. G. A., Schram, M. T., Colhoun, H. M., Hanssen, N. M. J., Niessen, H. W. M., Tarnow, L. and Schalkwijk, C. G. 2013. Methylglyoxal-derived AGE in diabetes. *Diabetologia* . **56**, 1845-1855.

Vistoli, G., De Maddis, D., Cipak, A., Zarkovic, N., Carini, M. and Aldini, G. 2013. AGEs and ALEs: Mechanisms of formation. *Free Radic. Res.* **47**, 3-27.

Vlassara, H. and Uribarri, J. 2014. Advanced glycation end products and diabetes. Curr. *Diabetes Rep.* **14**, 1-17.

Wang, Q., Zhou, J., Xiang, Z., Tong, Q., Pan, J., Wan, L. and Chen, J. 2019b. Anti-diabetic effects of Cassiae Semen extract. *J. Ethnopharmacol.* **239**, 111904.

Wu, Q., Tang, S., Zhang, L., Xiao, J., Luo, Q., Chen, Y. and Wang, C. 2020. Catechin inhibition of AGE formation. *Food Funct.* **11**, 5396-5408.

Yadav, N., Palkhede, J. D. and Kim, S. Y. 2023. Anti-glucotoxicity effect via inhibiting MGO-AGEs formation. *Int. J. Mol. Sci.* **24**.

Z, S., N, S., BM, R. and H, H. 2019. Aloe vera in metabolic syndrome: A review. *Phytother. Res.* **33**, 2649-2660.

Zari, S. T. and Zari, T. A. 2015. Medicinal plants for eczema. *J. Med. Plants Res.* **9**, 702-711.

Zhang, Z. and Li, K. 2018. Curcumin attenuates high glucose-induced inflammatory injury. *J. Diabetes Investig.* **9**, 731-740.