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Freeze-dried herbal *Kaempferia galanga* supplementation effectively modulates hyperglycemia-induced oxidative stress and apoptosis in diabetic BALB/c mice

Flavius Phrangsngi Nonglang¹, Revelbornstar Snaitang², Dhritiman Roy³ and Surya Bhan^{1*} 

Abstract

Background A dysfunction in insulin secretion or action leads to hyperglycemia. Hyperglycemia then causes the activation of pathways that result in increased production of reactive oxygen and nitrogen species (ROS/RNS) levels, ultimately causing oxidative stress. Oxidative stress overload then causes cellular damage and also promotes the increased activation of the apoptosis pathway inducing cell death. Thus, regulation of glucose homeostasis to prevent hyperglycemia is crucial. In this study, the potential protective effect of *Kaempferia galanga* herbal extract (KGE) on hyperglycemia-induced oxidative stress and apoptosis was investigated.

Result In this study, *Kaempferia galanga* (KG) herbal extracts, namely aqueous (KGA), ethanolic (KGE), methanolic (KGM), and chloroform (KGC), were tested for their antioxidant activity. In *in vitro* antioxidant assays, KG ethanolic extract (KGE) has the highest antioxidant activity out of all the extracts. High-performance thin layer chromatography phytochemical fingerprinting (HPTLC) analysis confirms that the presence of more antioxidant compounds in herbal KGE and ethyl-p methoxy cinnamate (EPMC) was the active phytochemical. Thus, KGE was chosen for *in vivo* studies. An intraperitoneal streptozotocin (STZ) administration produced a diabetic mouse model. *In vivo* herbal KGE treatment positively modulates SOD and CAT gene and protein expression in diabetic mice. Tissue protection from herbal KGE supplementation is supported by liver electron microscopy. In diabetic mice, herbal KGE supplementation reduces DNA fragmentation in the liver, kidney, pancreas, and heart by upregulating the gene and protein expression of anti-apoptotic BCL-2, inhibiting BAX expression, and ultimately inhibiting caspase-3 (CAS-3) expression. Herbal KGE supplementation in diabetic mice maintains insulin levels in serum and pancreas, indicating its protective role in preventing pancreatic damage or promoting β cell regeneration. Molecular docking analysis shows EPMC's high binding affinity for CAS-3, BAX, and BCL-2 compared to metformin suggesting that it may be responsible for modulating apoptotic protein expression.

Discussion Herbal KGE supplementation protects against diabetes-induced tissue damage and apoptosis by reducing hyperglycemia-induced oxidative stress and apoptosis, and EPMC may be the active component eliciting the effect.

Keywords Diabetes, *Kaempferia galanga*, Ethyl-p methoxy cinnamate, Oxidative stress, Apoptosis, HPTLC, Molecular docking

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Background

Diabetes is a chronic metabolic disease, which affects people worldwide. Absolute or relative insulin deficiency and/or action impairments result in persistent hyperglycemia, ultimately causing abnormalities in carbohydrate, protein, and lipid metabolism. Hyperglycemia-induced metabolic disturbances then cause micro and macro-vascular dysfunctions [1]. A recent prediction by the International Diabetes Federation (IDF) states that 536.6 million people worldwide, or one in ten adults were living with diabetes in 2021. By 2045, that number would rise to 783.2 million, representing a 12.2% increase in prevalence. Over 80% of people with diabetes live in low- and middle-income countries. Furthermore, diabetic complications accounted for about 6.7 million deaths [2].

Under normal physiological conditions, free radicals such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) have been shown to stimulate a variety of enzymatic cascades. Free radicals are physiologically produced by immune cells, metabolic processes, and other processes and are necessary for biological homeostasis [3]. However, chronic and persistent hyperglycemia leads to excess production of ROS/RNS and deregulation of ROS/RNS signaling pathways starts the development of diabetes mellitus [4]. Chronically increased glucose levels interfere with the ETC at complex III, causing coenzyme Q to oxidize molecular oxygen and produce increased superoxide anion leading to oxidative stress [5]. An increase in oxidative stress results in the accelerated oxidation of lipids, nucleic acids, proteins, and other cell components. Both type 1 and type 2 diabetes are associated with increased oxidative stress [6]. Oxidative stress may link many systems involved in diabetic complications like cardiovascular disease, neuropathy, nephropathy, and retinopathy [7].

High glucose causes a wide range of cellular reactions, but they all lead to functional alterations and frequently apoptotic cell death [1, 5]. Both *in vitro* and *in vivo* studies have reported that high glucose-induced tissue dysfunction and cell death may be caused by the activation of various proteins and signaling pathways that are involved in apoptosis [8, 9]. Apoptosis is a process of natural cell death that is necessary for all multicellular organisms to maintain homeostasis and normal development. Adverse biological effects, however, can result from both excessive and insufficient apoptotic cell death [5]. The process of apoptosis is regulated by key cellular proteins such as pro-apoptotic proteins (BAX, BAK, etc.) which activate apoptosis and also by anti-apoptotic proteins (BCL-2/BCL-xL, etc.) which inhibit apoptosis by inhibiting the activation of the caspases signaling cascade [5]. Elevated glucose causes oxidative stress which then triggers the increased acceleration of apoptosis by Bax-mediated

mitochondrial permeability and cytochrome-c release, while concurrently blocking BCL-2 proteins. After the mitochondria releases cytochrome-c, imitator caspases like CAS-9 become active, which, in turn, activates executioner CAS-3, which will finally execute the apoptotic process [10]

The rising demand for natural products, fuelled by the abundance of bioactive compounds in plants and their synergistic therapeutic effect, has driven research toward exploring natural antioxidants. These plant-derived compounds, like polyphenols, flavonoids, and carotenoids, not only possess potent antioxidant properties but also offer additional health benefits [11, 12]. Furthermore, natural antioxidants are generally perceived as safer, and more sustainable options compared to synthetic alternatives [13, 14]. Plants rich in these antioxidants play a vital role in alleviating oxidative stress-related conditions like diabetes mellitus [15].

Kaempferia galanga (*K. galanga*) one of the most important traditional Asian medicinal herbs, belonging to the Zingiberaceae family, exemplifies this concept, with reported antioxidant properties alongside various other pharmacological and therapeutic benefits [16]. In our previous studies, we observed that herbal *K. galanga* supplementation was able to alleviate hyperglycemia by modulating key proteins involved in insulin signaling, glycolytic, and gluconeogenic pathways [17]. Therefore, this study aims to comprehensively investigate the therapeutic efficacy of freeze-dried herbal *K. galanga* supplementation in mitigating hyperglycemia-induced oxidative stress and apoptosis. The study will focus on the effect of herbal *K. galanga* herbal extracts in modulating key oxidative stress markers (SOD, CAT), apoptotic proteins (BAX, BCL-2, CAS-3), crucial diabetic markers (insulin levels), tissue-protective effect in STZ-induced diabetic mice and also to elucidate the underlying mechanisms of action.

Methods

Preparation of *K. galanga* herbal extract

K. galanga rhizome was collected, and various herbal extracts, namely aqueous (KGA), ethanol (KGE), methanol (KGM), and chloroform (KGC), were prepared by lyophilization processes [16].

In vitro antioxidant activities

Ferric reducing antioxidant properties (FRAP)

The FRAP assay was done following the methodology of Benzie and Strain, with minor adjustments [18].

Total antioxidant assay (TAC)- Phosphomolybdenum assay

The TAC assay was done following the methodology of Prieto et al. with minor adjustments [19].

Phytochemical fingerprinting and in vitro antioxidant activity using HPTLC

The sample preparation and application were followed as per the protocol by Nonglang et al. [20] with slight modifications. 10 mg/mL concentration of various extracts of *K. galanga* and 1 mg/mL of standard reference (ascorbic acid, gallic acid, pyrogallol, quercetin, and EPMC) were prepared. After that, prepared samples were run on a TLC aluminum sheet silica gel 60 F 254 (Merck). Using a Linomat 5 sample applicator set at a speed of 150 nL/sec, 2 µL, 6 µL of plant extracts, and 2 µL of standard reference compounds was applied onto the plate.

Satisfactory resolution and separation of bands were obtained in the following solvent system: toluene/ethyl acetate/methanol/ formic acid in the ratio of 6:3:2.6:0.8 (v: v: v: v). The air-dried plates were examined under white light and in the ultraviolet light (254 nm and 366 nm) using a CAMAG® TLC Visualizer, and their R_f value was noted. Then derivatization of the plate with 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution was done to check the antioxidant activities and the R_f value of bands showing antioxidant activities were noted.

The derivatized plate was scanned at a spectrum speed of 100 nm/s using a deuterium lamp set to a 254 nm wavelength on a CAMAG® HPTLC Densitometer (Scanner 3). The peaks on the chromatogram were labeled with their R_f values.

Animal & experimental design

Swiss albino mice of Balb/C strain weighing around 22–28 g were utilized in the experimental study maintained in the animal room at an optimum temperature and fed with a standard mice diet and given water ad libitum. The Institutional Animal Ethics Committee (IAEC) approved the experimental protocols and methods used in the study.

Induction of diabetes

For diabetic induction in mice, STZ was used. Mice were starved overnight (but given water ad libitum) for approximately 8–10 h, and then STZ at a dose of 150 mg/kg b.w dissolved in 0.1 M cold citrate buffer (pH 4.5) was injected into the mice intraperitoneally. Then just after administration, the mice were fed and given 5% sucrose water overnight. Three days after the STZ injection, the FBGL was checked using a glucometer, and mice with FBGL above 200 mg/dL were considered diabetic and proceeded with the experiment [21].

Collection of samples

Following a 21-day course of therapy, blood was collected from the retro-orbital sinus. Special care was

taken by giving an anesthesia eye drop to reduce pain. The mice were then sacrificed ethically by administering a ketamine/xylazine cocktail (100 mg/10 mg). The tissues of study interest (liver, pancreas, kidney, and heart) and blood (serum was isolated) were collected and then stored at -80 °C.

Study on the effect of herbal KGE on oxidative stress

For the oxidative stress studies, mice were divided into four groups, each consisting of six mice, and the study was carried out for 21 days.

Group 1: Normal mice (NC).

Group 2: Diabetic mice (DC).

Group 3: Diabetic mice + 100 mg/kg b.w. of ascorbic acid (DA).

Group 4: Diabetic mice + 350 mg/kg b.w. of herbal KGE (DT).

Study on the effect of herbal KGE on apoptosis

For the apoptosis study, mice were divided into four groups, each consisting of six mice, and the study was carried out for 21 days.

Group 1: Normal mice (NC).

Group 2: Diabetic mice (DC).

Group 3: Diabetic mice + 100 mg/kg b.w. of metformin (DM).

Group 4: Diabetic mice + 350 mg/kg b.w. of herbal KGE (DT).

Study on the effect of herbal KGE on gene expressions by RT-PCR assay

Total RNA extraction and cDNA synthesis

Using Trizol Reagent (Sigma-Aldrich), total RNA was isolated from liver tissues as directed by the manufacturer. Using a Nanodrop spectrophotometer (Thermo Scientific), the concentration and purity of RNA were assessed by quantitation using the 260/230 nm absorbance ratio and the 260/280 nm absorbance ratio. cDNA iScript Reverse Transcription kit (Bio-Rad) was used to create first-strand cDNA from 400 ng of total RNA using the standard procedure. After that, the cDNA was kept at -80 °C for further use. The NCBI PubMed primer blast program was used to create and obtain real-time PCR nucleotide primers for *CAT*, *SOD*, *CAS-3*, *BCL-2*, *BAX*, and *GAPDH*. The following primer sequences were utilized as shown in Table S1 and Table S2 (Supplementary material 1).

Quantitative real-time PCR (qPCR)

Using Bio-Rad iTaq Universal SYBR Green chemistry, the qPCR was carried out in a Bio-Rad CFX96 Real-time PCR system (Bio-Rad, USA) to assess the pattern of expression of various genes under various experimental settings. 3.75 μ L of SYBR Green Mix, 100 μ M of each primer, 1 μ L of cDNA, and MilliQ H₂O were included in the 10 μ L reaction mixture. The assay parameters comprised the initial denaturation step for 30 s at 95 °C, 40 cycles for 2 s at 95 °C, and 30 s at 60 °C. The analysis was performed in triplicates. Melt-curve analysis was employed to verify that a solitary PCR product had been amplified. *GAPDH* was used as an endogenous reference gene to normalize the data, and the modified $\Delta\Delta$ CT method was used to calculate the relative m-RNA expression [22].

Study on the effect of herbal KGE on protein expressions by western blot assay

The excised liver tissues of mice from all groups were homogenized in 50 mM Tris HCl buffer (pH 7.4) containing 150 mM NaCl, 1% Triton X-100, 100 mM PMSE, 1 mM EDTA, and a protease inhibitor (Roche, Germany), and centrifuged at 12,000 g for 15 min at 4 °C. The tissue homogenates prepared from the acquired supernatants were utilized for biochemical analysis. The protein concentration of the tissue homogenate was examined using the Bradford method with slight modification [23]. Total protein from hepatic tissues was separated by SDS-PAGE (10% to 15%) and transferred to PVDF membranes (Millipore, USA). The membranes were treated overnight at 4 °C with primary antibodies as shown in Table S3 (Supplementary material 1). After three 10-min phosphate buffer saline-Tween 20 (PBST) washes, membranes were incubated with secondary antibodies for 2 h at room temperature. Finally, the protein expression bands were detected and quantified using an enhanced chemiluminescence detection system (Clarity™ Western ECL Substrate Bio-Rad, USA) in the Image Quant LAS 500 system (GE Healthcare Life Sciences, USA).

Terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick-end labeling (TUNEL) assay:

DNA fragmentation *in situ* was detected and performed using the fluorescein-based TUNEL assay kit (Roche, Switzerland) as per the given standardized protocol and principle and examined under a fluorescence microscope (TCS SP5, Leica, Germany).

Study on the effect of herbal KGE on insulin secretion:

For this study, mice were divided into five groups, each consisting of four mice, and the study was carried out for 21 days.

Group 1: Normal mice (NC).

Group 2: Diabetic mice (DC).

Group 3: Diabetic mice + 100 mg/kg b.w. of metformin (DM).

Group 4: Diabetic mice + 10 mg/kg b.w. of glipizide (DG).

Group 5: Diabetic mice + 350 mg/kg b.w. of herbal KGE (DT).

The study was performed using the Fine Test, Mouse Insulin (INS) ELISA Kit (Cat EM0260). The 96-well plate had pre-attached anti-INS antibodies. 100 μ L of standards and samples (serum and pancreas tissue homogenate) were added sequentially to the wells. Wash buffers removed unconjugated molecules after incubation. A biotin-conjugated anti-insulin antibody was added to bind the coated INS antibodies. Unbound conjugates were washed before adding HRP-Streptavidin. The HRP enzymatic reaction was visualized by adding TMB substrates following the third washing. A blue product was created by the reaction of TMB and HRP. Then, immediately, a stop solution was added. The sample's optical density (O.D.) was measured at 450 nm using a microplate reader (Bio-Rad, USA). The sample's insulin concentration was calculated using a standard curve. The OD450 value directly correlates to the insulin concentration.

Transmission electron microscopy studies (TEM):

For TEM studies, ultra-thin sections of liver tissues of various experimental groups were prepared following the method described by Hayat [24] with some modifications and viewed under the TEM instrument (JEM-100 CX II).

In-silico molecular docking studies:

Ligand and protein preparation

Ligands were collected from Pubchem database (<https://pubchem.ncbi.nlm.nih.gov/>). Proteins with protein data bank (PDB) codes 1F16 (BAX), 2W3L (BCL-2), and 3GJQ (CAS-3) were downloaded from (<https://www.rcsb.org/>). Using Discovery Studio Visualizer (v16.1.0.15350), the proteins were prepared by removing water molecules, co-crystal ligands, and unwanted chains.

Molecular docking

Molecular docking was investigated using PyRx (v0.8) to assess ligand–protein binding affinity and interactions,

with Autodock Vina providing binding energy predictions and ligand postures [25, 26]. Both protein and ligands were converted to PDBQT format (Protein Data Bank, Quaternary Format) for docking, which includes key information like polar hydrogen atoms, atom types, and partial charges. The functional binding sites of the proteins remain unknown and unclear; therefore, all binding sites were limited within the areas of probable docking pockets predicted by the DeepSite webserver (<https://www.playmolecule.com>). Briefly, 3D grid was generated with center ($x=2.81288015714$, $y=-4.04348130177$, $z=-4.30386172557$) for 1F16, center ($x=41.310871755$, $y=28.9845426277$, $z=-11.4220811305$) for 2W3L, and center ($x=28.4733883885$, $y=34.3125497754$, $z=41.9313357256$) for 3GJQ. The dimension of the grid box in the docking protocol was set to $x=25.0$, $y=25.0$, $z=25.0$ with an exhaustive value of 8. The ligand–protein interaction was analyzed using the PLIP (Protein–Ligand Interaction Profiler) webserver [27] and by PyMOL v2.5 (Educational version) [28].

Statistical analysis

The statistical tool Graph Pad Prism 9 was used to perform a one-way analysis of variance (ANOVA) followed by Tukey's post hoc test to examine differences between experimental groups. The data were presented as mean $\pm$ SEM. The statistical significance level was chosen at $p < 0.05$.

Results

In vitro antioxidant activity of the herbal *K. galanga* extracts

FRAP assay also shows that the herbal KGE has better antioxidant activity with 23.6 ± 1.60 mg AAE/g dry weight of the extract. On the other hand, KGM, KGA, and KGC have antioxidant activity of 9.75 ± 0.35 mg AAE/g dry wt. of extract, 5.1 ± 0.50 mg AAE/g dry wt. of extract, and 2 ± 0.40 mg AAE/g dry wt. of the extract, respectively (Table 1a).

TAC assays show that herbal KGE has better antioxidant activity with 70.80 ± 1.60 mg AAE/g dry wt. of the

extract. On the other hand, KGM, KGA, and KGC have antioxidant activity of 21.35 ± 1.85 mg AAE/g dry wt. of extract, 8.60 ± 2.50 mg AAE/g dry wt. of extract, and 4.10 ± 1.70 mg AAE/g dry wt. of the extract, respectively (Table 1b).

From the *in vitro* antioxidant analysis, it can be observed that herbal KGE has the most potent antioxidant activity as compared to the other extracts. This could be attributed to the better extraction capability of more flavonoid and phenolic compound content in herbal KGE.

HPTLC phytochemical profiling

As illustrated in Fig. 1, phytochemical fingerprinting and separation of the various *K. galanga* extracts were carried out using HPTLC analysis in conjunction with standard reference compounds, namely ascorbic acid, gallic acid, pyrogallol, quercetin, and EPMC. To separate the phytochemicals, different solvent system combinations were used. However, it was found that the solvent system namely toluene: ethyl acetate: methanol: formic acid in the ratio of 6:3:2.6:0.8 was the solvent combination that produced the best separation. Ascorbic acid, gallic acid, pyrogallol, quercetin, ethyl-p-methoxy cinnamate (EPMC), and *K. galanga* extracts in the order of KGE, KGM, KGA, and KGC were applied on the TLC plates. The compound EPMC was found to be present in the herbal KGE, KGM, and KGC at an Rf value of 0.88.

Additionally, derivatization with the DPPH radical solution of the TLC plate was done to see the separated compounds' antioxidant activity compared with the standard reference (Fig. 2). It was observed that all the extracts, namely KGE, KGM, KGA, and KGC, contain phytochemicals that possess antioxidant activity. However, herbal KGE possesses more phytochemicals that exhibited antioxidant potential as compared to the other extracts, as evident from the change in color of purple DPPH radical to yellow stable compound upon reduction by an antioxidant. Thus, based on the *in vitro* antioxidant activity and the HPTLC-DPPH analysis, herbal KGE was chosen as the potent extract for further *in vivo* studies. Further, scanning of the peak chromatogram after DPPH derivatization shows the various compounds' antioxidant capacity (Fig. 3) and the Rf value of compounds and standards having antioxidant activity has been detailed as shown in Table 2.

Effect of herbal KGE on insulin content

The insulin level in the serum and pancreatic tissue homogenates was analyzed as shown in Fig. 4. It was observed that the serum insulin level in the DC mice group was significantly decreased by 4.14-fold ($p < 0.01$) as compared to the NC mice group. On the contrary, the

Table 1 FRAP and TAC of the various extracts of *K. galanga* expressed in mg AAE/ g dry weight of extract

Plant name/standards	mg AAE/g dry weight of extract	
	FRAP	TAC
Herbal KGA	9.75 ± 0.35	21.35 ± 1.85
Herbal KGE	23.6 ± 1.60	70.80 ± 1.60
Herbal KGM	5.1 ± 0.50	8.60 ± 2.50
Herbal KGC	2 ± 0.40	4.10 ± 1.70

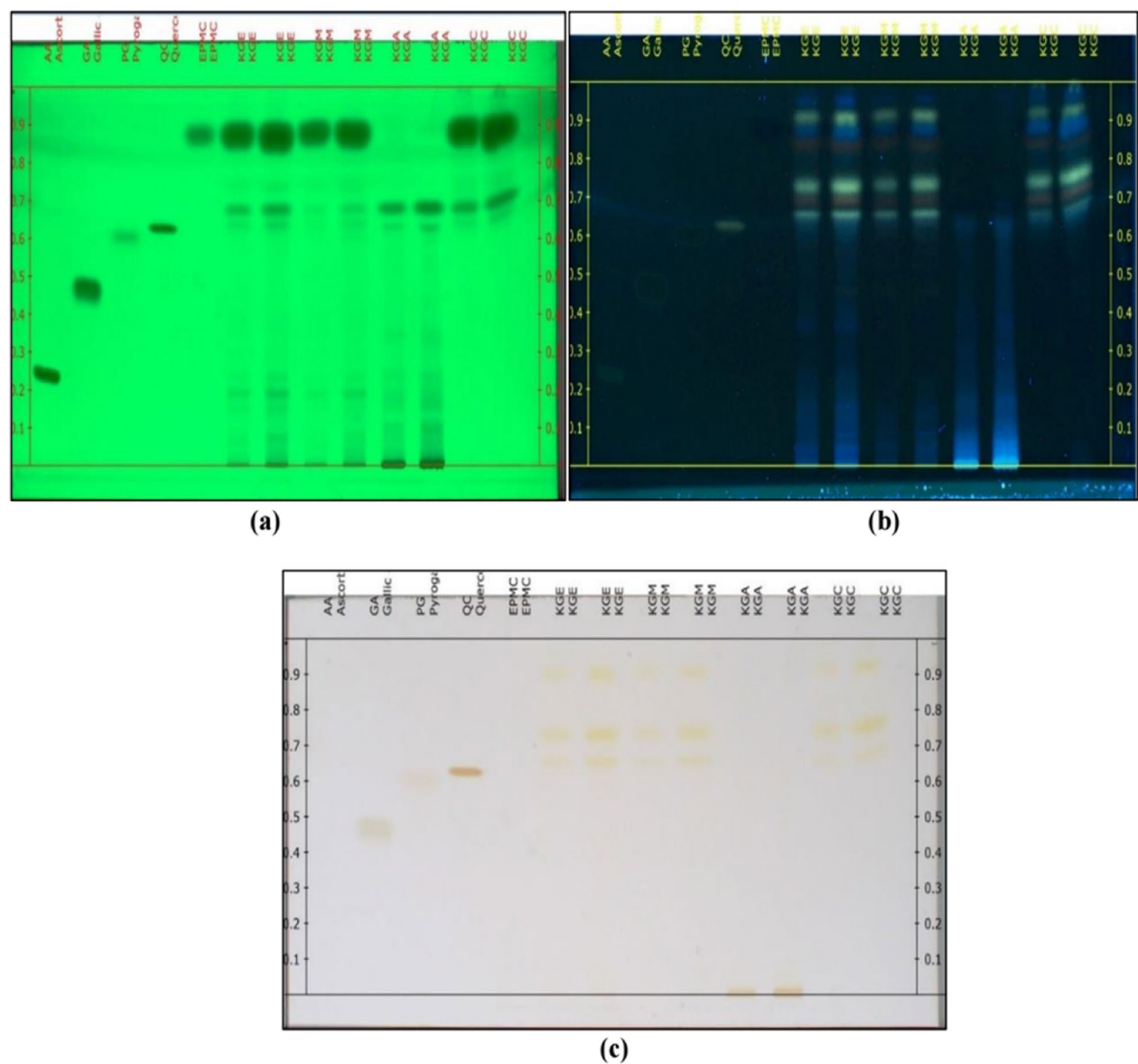


Fig. 1 Phytochemical separation and profiling: **a** 254 nm developed, **b** 366 nm developed, **c** white developed

diabetic mice group treated with standard drugs, DM and DG showed a significant increase in insulin level by 3.11-fold ($p < 0.05$) and 3.48-fold ($p < 0.01$), respectively, as compared to the DC mice group. Furthermore, the herbal KGE-supplemented mice group (DT) showed a significant increase in insulin level by 3.52-fold ($p < 0.01$) as compared to the DC mice group.

Similarly, it was observed that the pancreas tissue insulin level in the DC mice group was decreased by 0.7-fold as compared to the NC mice group. On the other hand, the diabetic mice group treated with

standard drugs, DM and DG, showed an increase in insulin level by 0.14-fold and 0.39-fold, respectively, as compared to the DC mice group. Furthermore, the diabetic mice group treated with herbal KGE (DT) showed an increase in insulin level in the pancreas tissue by 0.45-fold as compared to the DC mice group.

Effect of herbal KGE on SOD protein and gene expression in various experimental groups

The protein and gene expression of SOD was analyzed, as shown in Fig. 5.

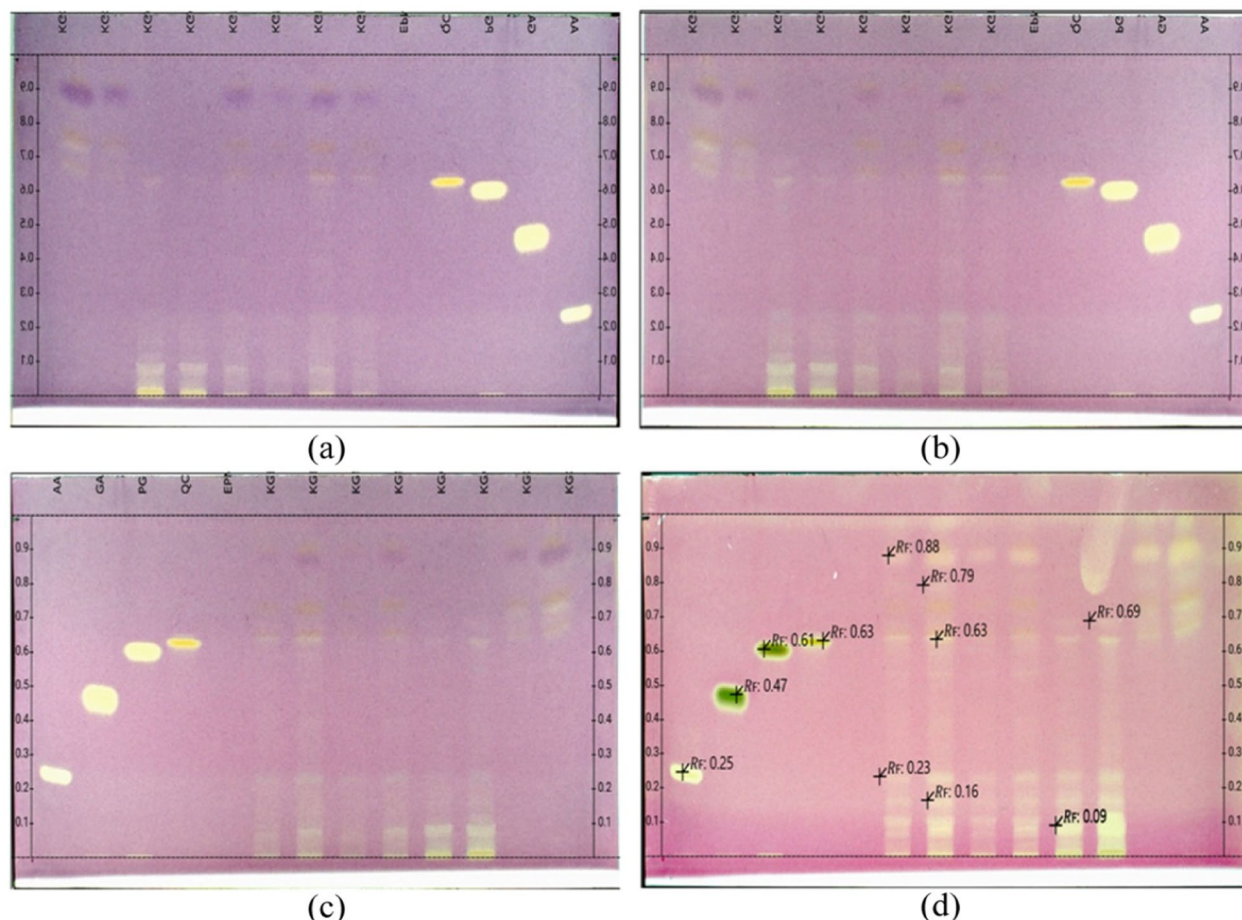


Fig. 2 Antioxidant profiling analysis-DPPH derivatization: **a** 0 min, **b** 30 min, **c** 60 min, **d** 24-h

(a) Protein expression studies

SOD protein expression was highly downregulated in the DC mice group, with a significant decrease of 0.33-fold ($p < 0.001$) against the NC mice group. However, DA and DT mice groups showed a significant increase of 0.22-fold ($p < 0.01$) and 0.21-fold ($p < 0.01$), respectively, in SOD protein expression as compared to the DC mice group (Fig. 5a and b).

(b) Gene expression studies

SOD gene expression was highly downregulated DC mice group with a significant decrease of 0.67-fold ($p < 0.001$) against the NC mice group. However, DA and DT mice groups showed a significant increase of 0.40-fold ($p < 0.01$) and 0.23-fold ($p < 0.01$), respectively, in SOD gene expression as compared to the DC group (Fig. 5c).

Effect of herbal KGE on CAT protein and gene expression in various experimental groups:

The protein and gene expression of CAT was analyzed as shown in Fig. 6.

a) Protein expression studies

CAT protein expression was highly downregulated in the DC mice group with a significant decrease of 0.42-fold ($p < 0.001$) against the NC group. However, the DA and DT mice group showed a significant increase of 0.31-fold ($p < 0.001$) and 0.21-fold ($p < 0.001$), respectively, in CAT protein expression as compared to the DC group (Fig. 6a and b).

b) Gene expression studies

CAT gene expression was significantly downregulated in the DC group, with a significant decrease of 0.68-fold ($p < 0.001$) against the NC mice group. However, DA and DT mice groups showed a significant increase of 0.33-fold ($p < 0.05$) and 0.28-fold ($p < 0.05$) in CAT gene expression as compared to the DC group (Fig. 6c).

Effect of herbal KGE on CAS-3 protein and gene expression in various experimental groups:

The protein and gene expression of CAS-3 was analyzed as shown in Fig. 7.

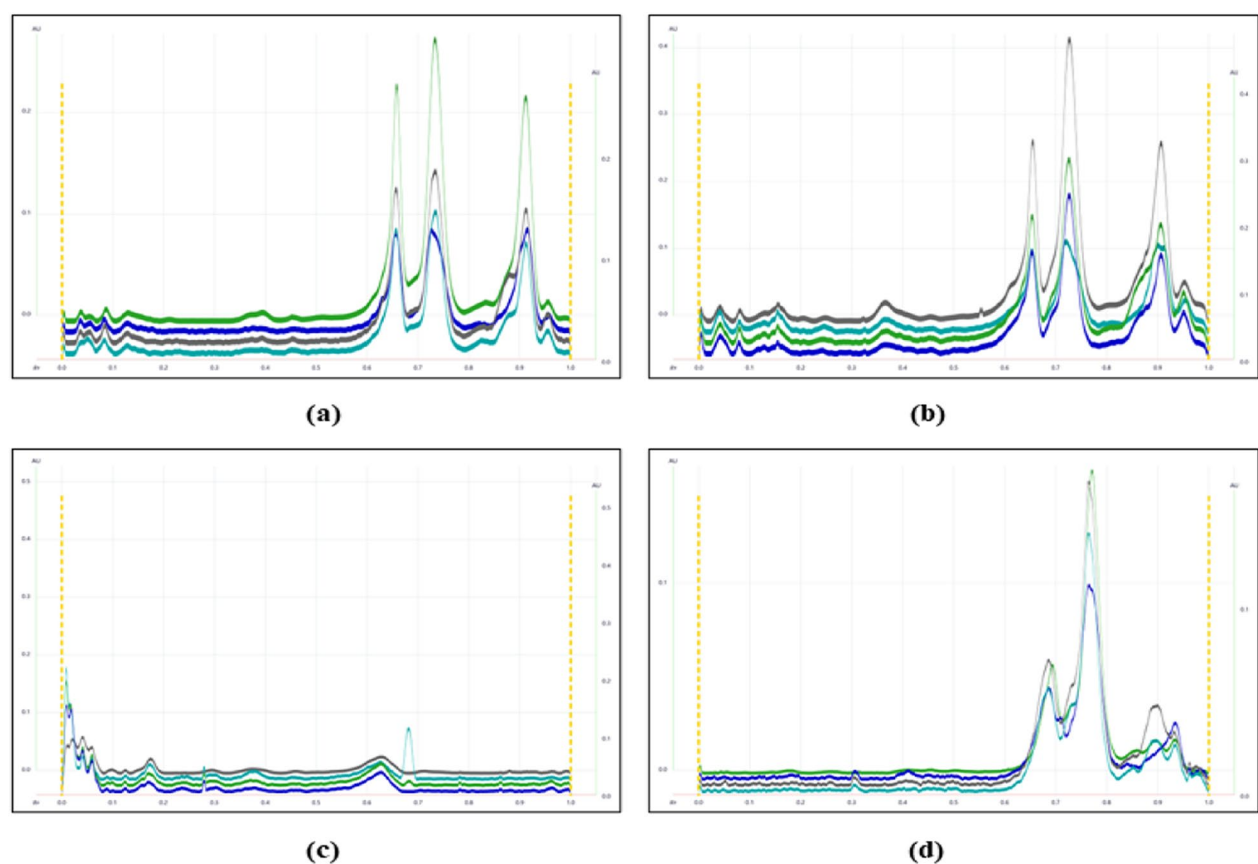


Fig. 3 Antioxidant peak chromatogram: **a** KGE, **b** KGM, **c** KGA, **d** KGC

Table 2 Separated compounds in various herbal extracts and standards with their Rf value

	Herbal <i>K. galanga</i>				Standards				
	KGE	KGM	KGA	KGC	AA	GA	QUE	PYR	EPMC
White	0.66, 0.73, 0.88	0.66, 0.73, 0.88	0.01	0.66, 0.73, 0.88	0.23	0.46	0.61	0.64	0.88
254 nm	0.19, 0.63, 0.69, 0.88	0.19, 0.63, 0.69, 0.88	0.63, 0.69	0.63, 0.69, 0.88	0.23	0.46	0.61	0.64	0.88
366 nm	0.03, 0.08, 0.16, 0.23, 0.36, 0.63, 0.66, 0.69, 0.73, 0.84, 0.88, 0.91, 0.94	0.03, 0.08, 0.16, 0.66, 0.69, 0.73, 0.84, 0.88, 0.91, 0.94	0.03, 0.08, 0.18, 0.63, 0.66	0.63, 0.66, 0.69, 0.73, 0.84, 0.88, 0.91, 0.94	0.23	0.46	0.61	0.64	0.88
DPPH	0.03, 0.08, 0.16, 0.23, 0.65, 0.69, 0.72, 0.79, 0.88	0.03, 0.08, 0.16, 0.23, 0.63, 0.65, 0.69, 0.79, 0.88	0.05, 0.09, 0.16, 0.23, 0.63, 0.65, 0.69	0.63, 0.69, 0.73, 0.79, 0.88	0.23	0.46	0.61	0.64	0.88

a) Protein expression studies

CAS-3 protein expression was highly upregulated in the DC mice group, with a significant increase of 0.64-fold ($p < 0.001$) against the NC group. However, the DM and DT mice groups showed a significant decrease of 0.59-fold ($p < 0.01$) and 0.36-fold ($p < 0.05$), respectively, in CAS-3 protein expression as compared to the DC group (Figs. 7a and b).

b) Gene expression studies

The gene expression shows that CAS-3 was highly upregulated in the DC with a significant increase of 0.83-fold ($p < 0.001$) against the NC group. However, the DM and DT mice group showed a significant decrease of 0.5-fold ($p < 0.05$) and 0.69-fold ($p < 0.01$) in CAS-3 gene expression as compared to the DC mice group (Fig. 7c).

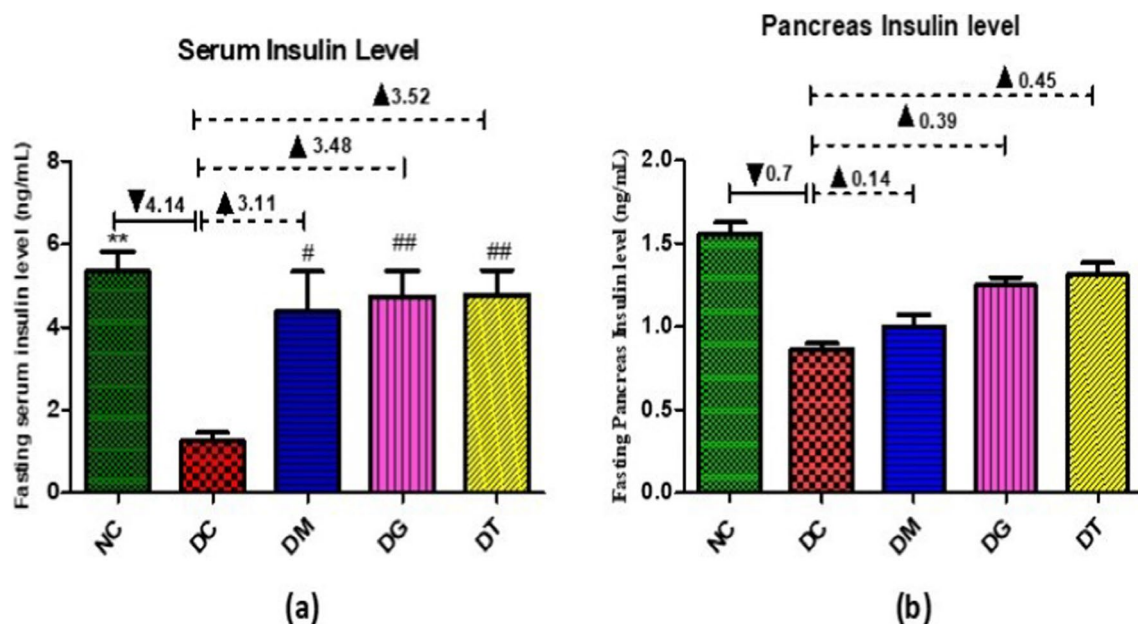


Fig. 4 **a** Serum insulin level, **b** Pancreas insulin level. Comparison between NC, DC, DM, DG, and DT mice groups. Values are expressed as the mean $\pm$ SEM; $n=4$. Significance difference: ** $p < 0.01$ of DC against the NC group; # $p < 0.05$, ## $p < 0.01$, of DM, DG, and DT against the DC group

Effect of herbal KGE on BCL-2 protein and gene expression in various experimental groups

The protein and gene expression of BCL-2 was analyzed, as shown in Fig. 8.

a) Protein expression studies

BCL-2 protein expression was highly downregulated in the DC mice group, with a significant decrease of 0.47-fold ($p < 0.001$) against the NC group. However, the DM and DT mice group showed a significant increase of 0.26-fold ($p < 0.001$) and 0.29-fold ($p < 0.001$), respectively, in BCL-2 protein expression as compared to the DC mice group (Figs. 8a and b).

b) Gene expression studies

BCL-2 gene expression was highly downregulated in the DC mice group with a significant decrease of 0.86-fold ($p < 0.001$) against the NC mice group. However, the DM and DT mice group showed a significant increase of 0.36-fold ($p < 0.001$) and 0.31-fold ($p < 0.01$) in BCL-2 gene expression as compared to the DC mice group (Fig. 8c).

Effect of herbal KGE on BAX protein and gene expression in various experimental groups:

The protein and gene expression of BAX was analyzed as shown in Fig. 9.

a) Protein expression studies

BAX protein expression was highly upregulated in the DC mice group with a significant increase of 4.04-fold ($p < 0.001$) against the NC mice group. However, DM and DT showed a significant decrease of 2.14-fold ($p < 0.001$) and 3.48-fold ($p < 0.001$), respectively, in BAX protein expression as compared to the DC mice group (Figs. 9a and b).

b) Gene expression studies

BAX gene expression was highly upregulated in the DC mice group with a significant increase of 0.93-fold ($p < 0.01$) against the NC mice group. However, the DM and DT mice group showed a significant decrease of 0.62-fold ($p < 0.05$) and 0.5-fold ($p < 0.05$) in BAX gene expression as compared to the DC mice group (Fig. 9c).

TUNEL assay studies on the various tissues to check the apoptotic index in various experimental groups:

Kidney

The TUNEL analysis of the kidney tissue was done and it was found that in the DC mice group, the apoptotic index significantly increased by 10.03-fold ($p < 0.001$) compared to the NC mice group. In contrast, the apoptotic index in the DM and DT mice group has decreased significantly by 6.7-fold ($p < 0.001$) and 6.41-fold ($p < 0.001$), respectively, compared to the DC mice group (Fig. 10).

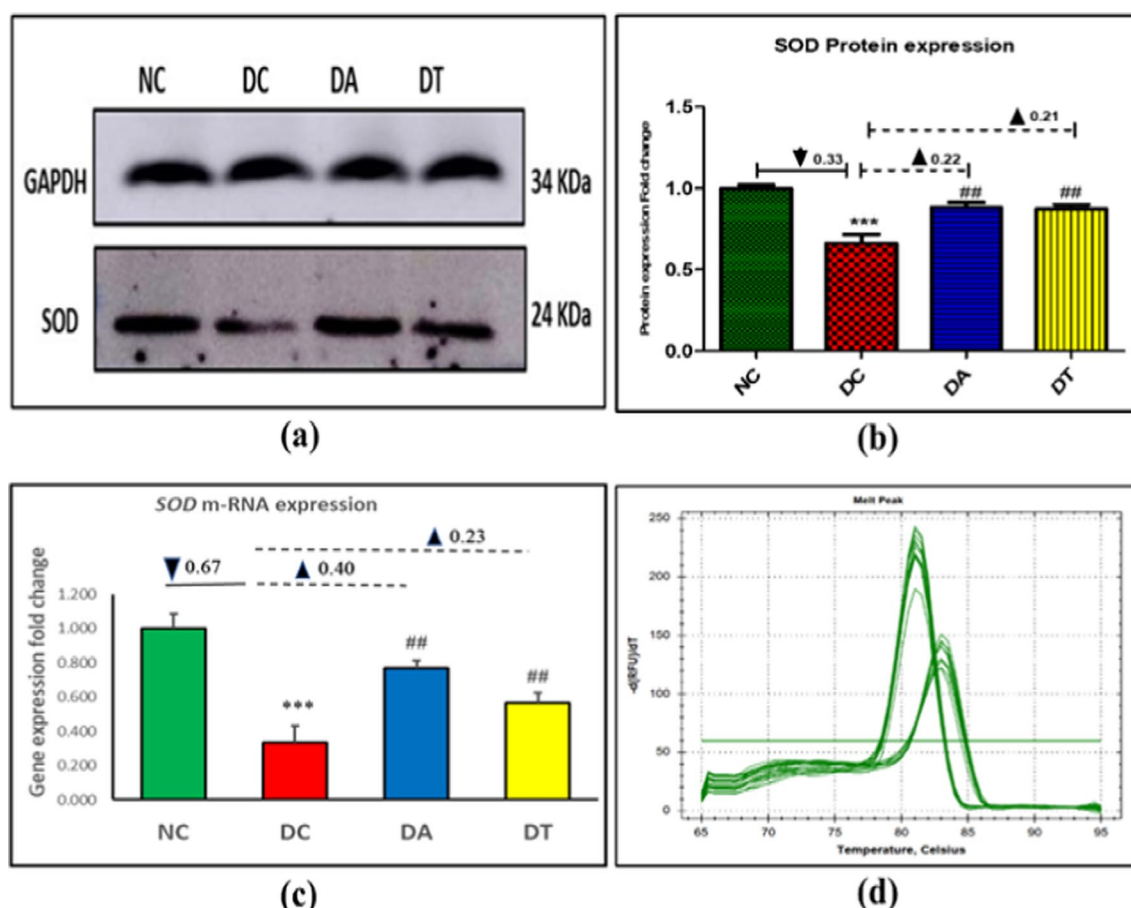


Fig. 5 **a** Comparison of the SOD protein expression between NC, DC, DA, and DT mice groups. **b** Densitometric analysis corresponding to the fold change after normalization with GAPDH. **c** SOD m-RNA gene expression fold change between various treatment groups after normalization with GAPDH. **d** Melt peak of SOD and GAPDH. Values are expressed as the mean $\pm$ SEM; $n = 3$. Significance difference: *** $p < 0.001$ of DC against the NC group; ## $p < 0.01$ of DA and DT against the DC group

Liver

The TUNEL analysis of the liver tissue was done, and it was found that in the DC mice group, the apoptotic index significantly increased by 4.91-fold ($p < 0.001$) as compared to the NC mice group. In contrast, the apoptotic index in the DM and DT has decreased significantly by 4.18-fold ($p < 0.001$) and 3.72-fold ($p < 0.001$), respectively, in comparison with the DC mice group (Fig. 11).

c) Pancreas

The TUNEL analysis of the pancreas tissue was done, and it was found that in the DC mice group, the apoptotic index significantly increased by 3.57-fold ($p < 0.01$) as compared to the NC mice group. In contrast, the apoptotic index in the DM and DT mice group has decreased significantly by 2.7-fold ($p < 0.05$) and 2.43-fold ($p < 0.05$), respectively, in comparison with the DC mice group (Fig. 12).

d) Heart

The TUNEL analysis of the heart tissue was done, and it was found that in the DC mice group, the apoptotic index significantly increased by 4.87-fold ($p < 0.001$) as compared to the NC mice group. In contrast, the apoptotic index in the DM and DT mice group has decreased significantly by 4.17-fold ($p < 0.001$) and 3.84-fold ($p < 0.001$), respectively, in comparison with the DC mice group (Fig. 13).

Transmission electron microscopy studies of the liver:

A typical hepatocyte ultrastructure was seen in the NC mice group, with a round nucleus that included scattered chromatin and prominent nucleoli. The DC mice group however showed a deformed nucleus that includes nearly dissolved chromatin and nucleoli. On the contrary herbal KGE supplementation restored hepatocytes morphology as observed by the presence of a round nucleus and

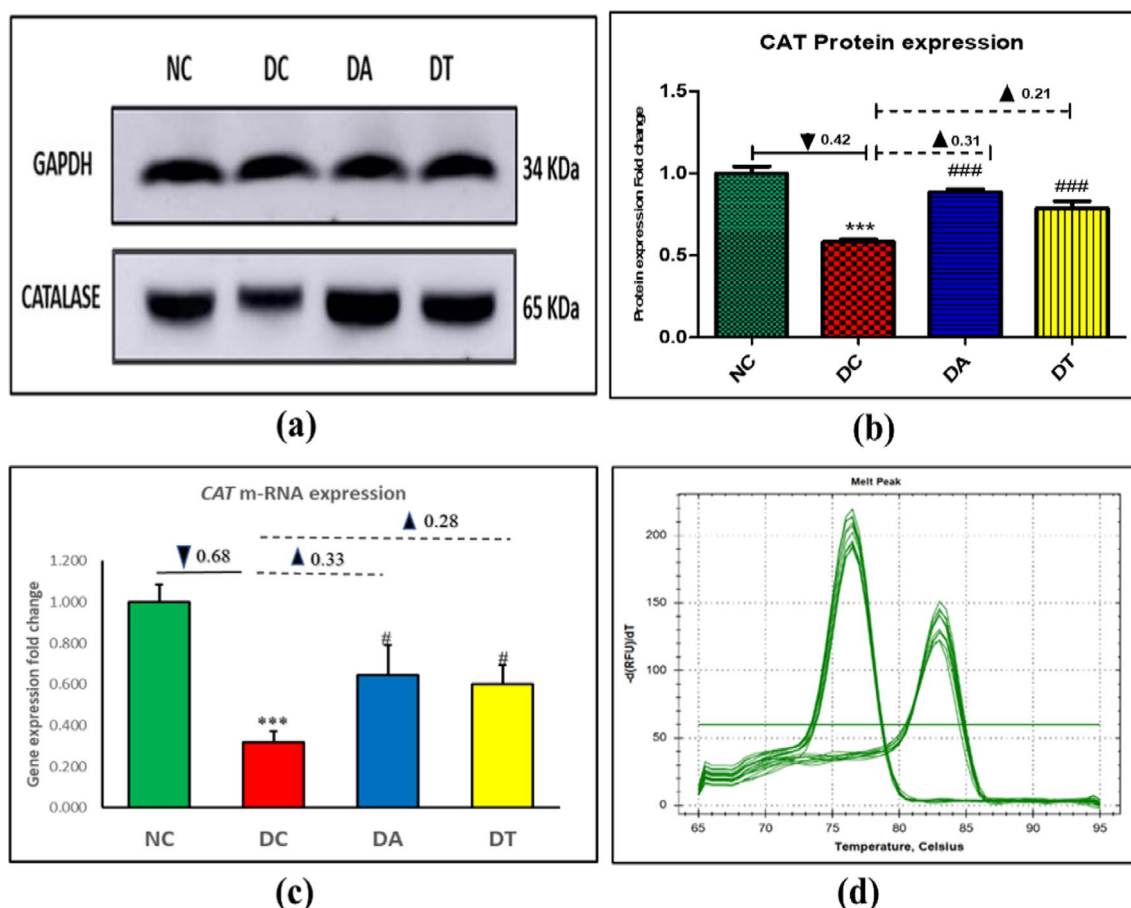


Fig. 6 **a** Comparison of the CAT protein expression between NC, DC, DA, and DT mice groups. **b** Densitometric analysis corresponding to the fold change after normalization with GAPDH. **c** CAT m-RNA gene expression folds change between various treatment groups after normalization with GAPDH. **d** Melt peak of CAT and GAPDH. Values are expressed as the mean $\pm$ SEM; $n = 3$. Significance difference: *** $p < 0.001$ of DC against the NC group; # $p < 0.05$, ### $p < 0.001$ of DA and DT against the DC group

finely visible nucleoli. This effect was comparable to that of ascorbic acid and metformin (Fig. 14).

Molecular Docking:

In-silico studies using molecular docking to determine the binding affinity of standard metformin drug and the bioactive compound found in herbal KGE, i.e., EPMC, have been done as detailed in Table 3. The binding affinity of EPMC with BAX, BCL-2, and CAS-3 was found to be -4.9 kcal/mol, -5.4 kcal/mol, and -4.5 kcal/mol, respectively, while the binding affinity of metformin with BAX, BCL-2, and CAS-3 was found to be -4.0 kcal/mol, -4.0 kcal/mol, and -3.7 kcal/mol, respectively. The 2D diagram showing the amino acid binding residue is shown in Fig. 15, 16, 17. The 3D diagram is shown in the Supplementary material 1 (Figure S1a-S1c).

Both metformin and EPMC form polar contacts such as hydrogen bonds and salt bridges with proteins, which is strong; in some cases, additional contacts and

interactions for EPMC are seen, which depicts a stronger binding affinity of EPMC than metformin with the proteins. Overall, the binding energy calculation of EPMC is much lesser than metformin for all the apoptotic proteins and, therefore, can bind and modulate them much more efficiently (activation or inhibition), benefiting in anti-apoptotic behavior as evidenced by *in vitro* and *in vivo* studies. Thus, EPMC might be the bioactive component responsible for electing the antihyperglycemic and anti-apoptotic effects in diabetic mice.

Discussion

Chronic and persistent hyperglycemia is associated with the development of diabetic complications due to the production of reactive species (ROS/RNS) leading to oxidative stress, and cellular death [29]. In our previous study, the administration of herbal KGE supplementation was found to lower blood glucose levels and reduce altered HbA1c levels in STZ-induced diabetic mice. This

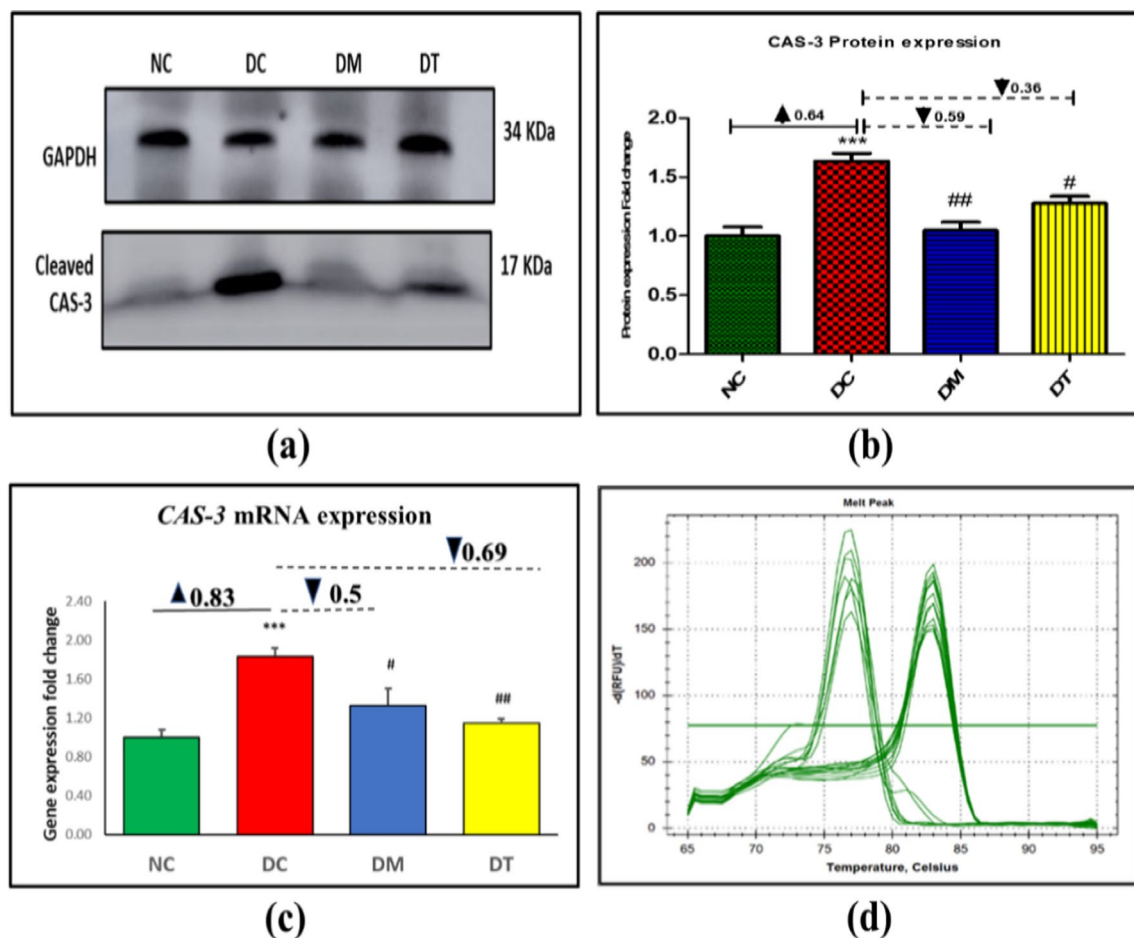


Fig. 7 **a** Comparison of the CAS-3 protein expression between the NC, DC, DM, and DT mice groups. **b** Densitometric analysis corresponding to the fold change after normalization with GAPDH. **c** CAS-3 m-RNA gene expression fold change between various treatment groups after normalization with GAPDH. **d** CAS-3 and GAPDH melt peak. Values are expressed as the mean $\pm$ SEM; $n = 3$. Significance difference: *** $p < 0.001$ of DC against the NC group; # $p < 0.05$, ## $p < 0.01$ of DM and DT against the DC group

was achieved by modulating the expression of genes involved in various metabolic pathways via stimulation of the expression of glucokinase and AKT/PKB and inhibiting the expression of glycogen synthase kinase-3 (GSK-3) and phosphoenol pyruvate carboxykinase (PEPCK) [17]. Therefore, in this study, we further investigate the protective effect of herbal KGE supplementation against oxidative stress and apoptosis in STZ-induced diabetic mice and the mechanistic pathway by which it elicits its effects (Fig. 18).

Research has shown that oxidative stress is linked to nearly every aspect that contributes to diabetes and its complications, indicating the significance of antioxidant therapy as an alternative treatment for this disease [15, 30]. The production of ROS/RNS is one of the earliest visible reactions of a cell to a high glucose challenge [31]. When ROS/RNS production exceeds the body's antioxidant defenses, cellular membranes, proteins, RNA, and

DNA can be damaged. This alters ROS/RNS signaling pathways and causes increased inflammatory responses, cell dysfunction, and then cell death [32]. Thus, eliminating ROS/RNS with exogenous antioxidant supplementation or improving the endogenous antioxidant enzyme system may help ameliorate oxidative stress-induced diabetes. Our preliminary *in vitro* analysis found that the herbal extracts of *K. galanga*, namely KGA, KGE, KGM, and KGC, showed good antioxidant activity, with herbal KGE having the strongest antioxidant capacity. Furthermore, HPTLC-DPPH profiling analysis in the various herbal extracts revealed the separation of various compounds having antioxidant activity, with the herbal KGE extracts containing more antioxidant compounds, explaining its stronger free radical scavenging activity.

Chronic hyperglycemia disrupts the electron transport chain (ETC) and causes its dysfunction, leading to increased molecular oxygen oxidation to form increased

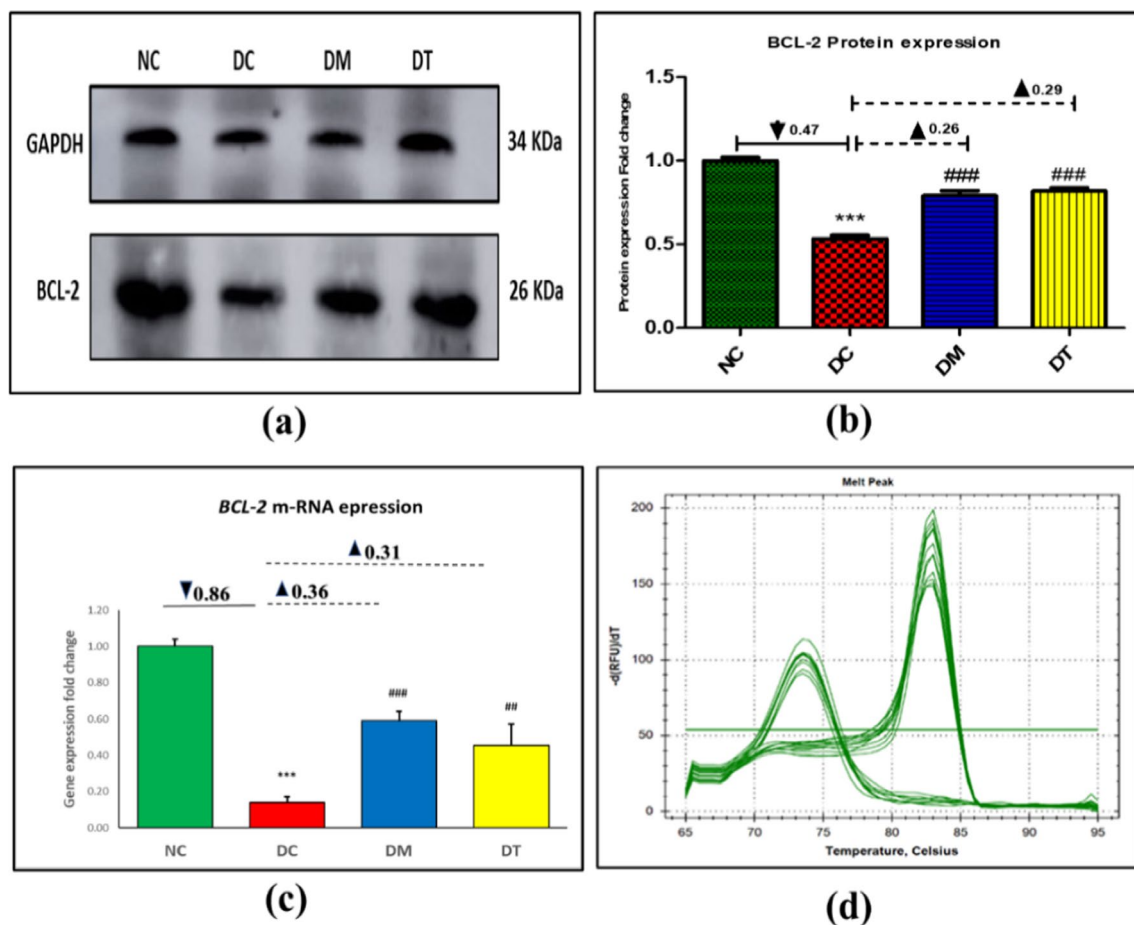


Fig. 8 **a** Comparison of the BCL-2 protein expression between NC, DC, DM, and DT mice groups. **b** Densitometric analysis corresponding to the fold change after normalization with GAPDH. **c** BCL-2 m-RNA gene expression fold change between various treatment groups after normalization with GAPDH. **(d)** BCL-2 and GAPDH melt peak. Values are expressed as the mean $\pm$ SEM; $n=3$. Significance difference: *** $p < 0.001$ of DC against the NC group; ## $p < 0.01$, ### $p < 0.001$ of DM and DT against the DC group

superoxide radicals [33]. Increased superoxide radical formation ultimately overwhelms the endogenous antioxidant enzyme defense system such as SOD and CAT leading to oxidative stress [34]. Furthermore, chronic hyperglycemia causes increased glycation of these endogenous antioxidant enzymes, ultimately disrupting their native conformation and causing their decreased activities [35]. This further exacerbates the free radical production ultimately leading to more oxidative stress. In our *in vivo* antioxidant analysis, diabetic mice had decreased CAT and SOD protein and gene expression. However, supplementation of the diabetic mice with standard ascorbic acid and herbal KGE significantly restored CAT and SOD gene and protein expression. Thus, herbal KGE provides exogenous antioxidant compounds as observed in our *in vitro* phytochemical profiling and also improves endogenous antioxidant enzyme system function, making it a promising antioxidant therapeutic adjuvant in diabetes treatment.

Apoptosis is a programmed process of cell death that occurs when the mitochondrial cytochrome-c-stimulated CAS-3 pathway is activated due to increased production of ROS/RNS induced by high glucose levels [36]. Increased ROS/RNS then cause damage to the DNA, resulting in elevated expression of poly (ADP-ribose) polymerase-1 (PARP-1) to repair the damaged DNA. However, in this process, PARP-1 increased activity leads directly to NAD^+ depletion and subsequently ATP depletion, which ultimately causes cell death [37]. In addition, ROS/RNS stimulates the expression of the p-53 protein, which can trigger the pro-apoptotic agent BAX/BAK and suppress the anti-apoptotic agent BCL-2/BCL-XL. This ultimately promotes the advancement of the apoptotic process and activates the downstream executioner caspase, such as CAS-3, 6, and 7 [38]. CAS-3 can then induce the activation of the specific nuclease caspase-3-activated DNase (CAD), which, upon entering the nucleus, can

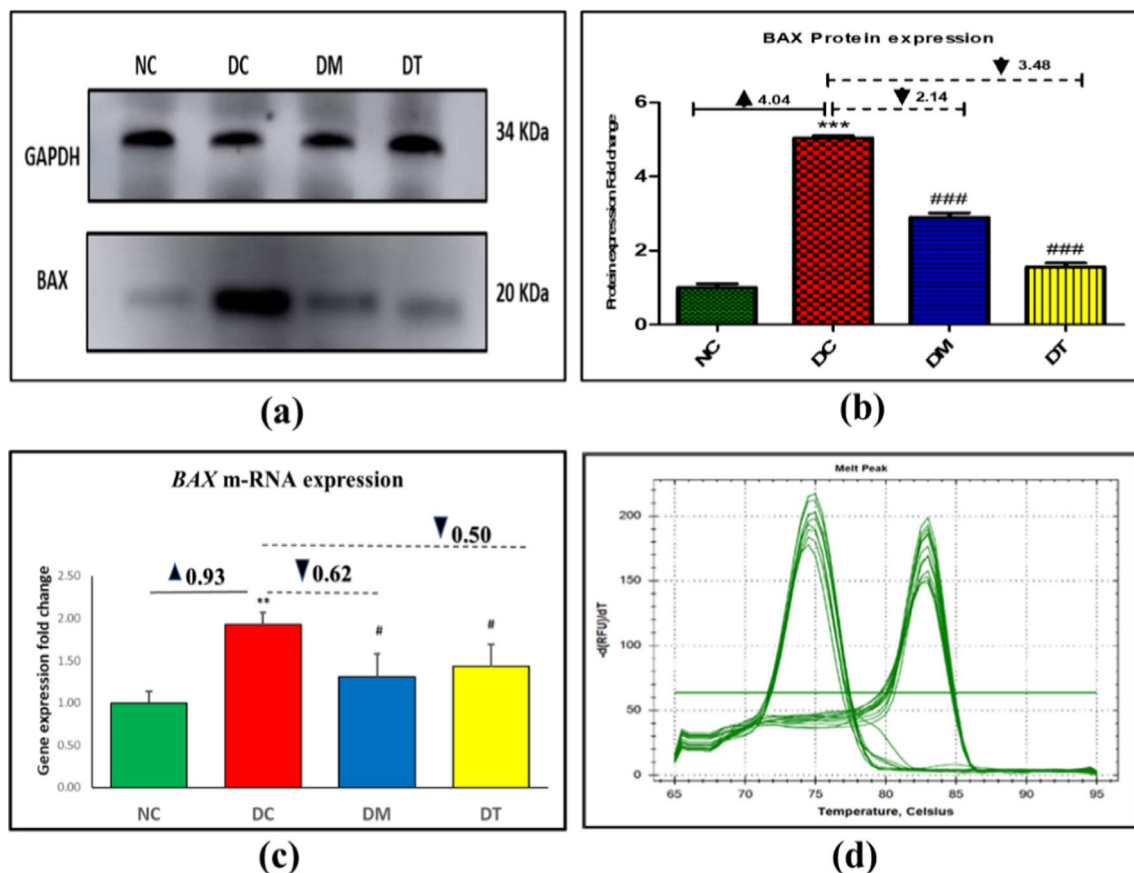


Fig. 9 **a** Comparison of the BAX protein expression between NC, DC, DM, and DT mice groups. **b** Densitometric analysis corresponding to the fold change after normalization with GAPDH. **c** BAX m-RNA gene expression folds change between various treatment groups after normalization with GAPDH. **(d)** Melt peak of BAX and GAPDH. Values are expressed as the mean $\pm$ SEM; $n = 3$. Significance difference: *** $p < 0.001$, ** $p < 0.01$ of DC against the NC group; # $p < 0.05$, ### $p < 0.001$ of DM and DT against the DC group

cause DNA fragmentation [39]. Research conducted on rats and mice has shown that the apoptotic process is characterized by an upregulation of caspase-3/9, BAX, and the BAX/BCL-2 ratio, along with a downregulation of BCL-2 [9]. In our study, it was observed that in diabetic mice, there is a significant increase in the gene and protein expression of pro-apoptotic BAX and a significant decrease in the anti-apoptotic BCL-2 gene and protein expression, indicating an increase in apoptotic signaling. Furthermore, due to the increase in oxidative stress and inflammatory pathways, there is an increase in the gene and protein expression of the executioner caspase-3 gene and protein expression. This causes an increase in apoptotic cell death in various tissues. However, supplementation of herbal KGE in diabetic mice significantly downregulated the gene and protein expression of BAX and simultaneously upregulated the gene and protein expression of BCL-2. Also, the herbal KGE supplementation significantly downregulated the gene and protein expression of CAS-3. It was observed

that the anti-apoptotic activity of herbal KGE is comparable to that of standard metformin drugs.

The fragmentation of the double-stranded genomic DNA by endonucleases, such as caspase-activated DNase (CAD) resulting in tiny nucleosomal fragments with unattached 3'-OH groups at their ends, is a feature shared by both the early and late stages of apoptosis. To further analyze the anti-apoptotic effect of herbal KGE, the TUNEL assay in various tissues of various experimental groups was performed. The TUNEL assay utilizes a unique DNA polymerase; terminal deoxynucleotidyl transferase which identifies DNA fragmentation by marking the unbound 3'-OH termini [40]. Terminal deoxynucleotidyl transferase (TdT) facilitates the incorporation of appropriately labeled (fluorescein) deoxynucleotide triphosphates (typically dUTP analogs) into a single-stranded sequence that possesses free 3'-OH terminus, in a manner that is not dependent on a template, which can then be detected when visualized under a fluorescence microscope [41, 42].

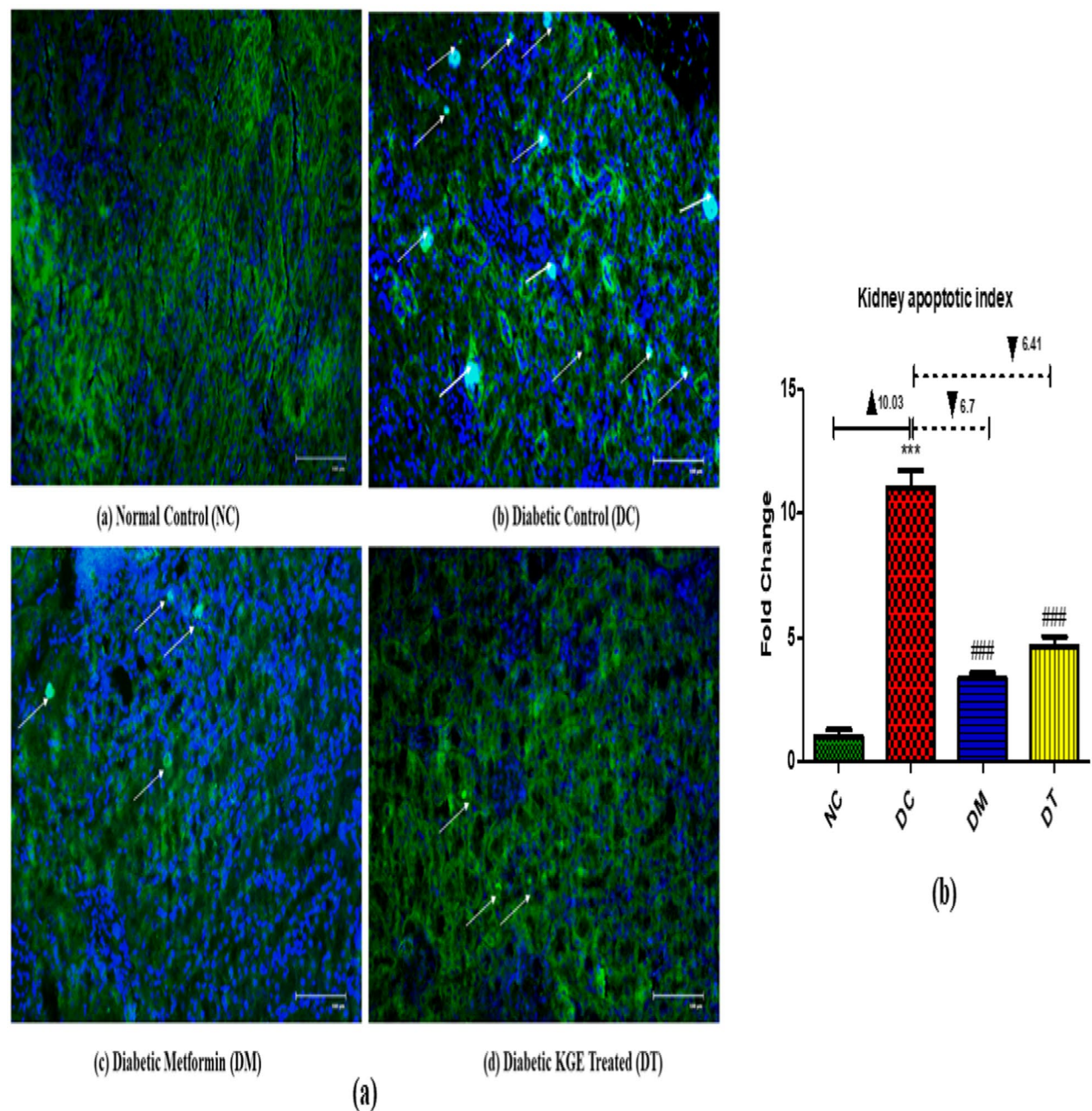


Fig. 10 **a** TUNEL assay of the kidney in NC, DC, DM, and DT mice groups. **b** Apoptotic index. Values are expressed as the mean $\pm$ SEM; $n=3$. Significance difference: *** $p < 0.001$ of DC against the NC group; ### $p < 0.001$ of DM and DT against the DC group. Green Spots: Fluorescein dye labeling of the fragmented DNA indicating apoptotic DNA fragmentation; Blue Spots: DAPI dye labeling of the nucleus

TUNEL assay depicted that the green fluorescence spot indicates fluorescein labeling of the fragmented DNA, while the blue fluorescence spot indicates DAPI labeling of the nucleus. In our study, the TUNEL assay shows that an upregulated apoptotic index (increased green fluorescence spots) was detected in the kidney, liver, pancreas, and heart tissue of diabetic mice as

shown in Fig. 10, 11, 12, 13, respectively. In contrast, it was noted that the supplementation of herbal KGE and metformin to diabetic mice resulted in a significant reduction of the apoptotic index (reduced green fluorescent spots) in all the tissues. Thus, the use of herbal KGE supplementation was successful in reducing oxidative stress and ultimately preventing the acceleration

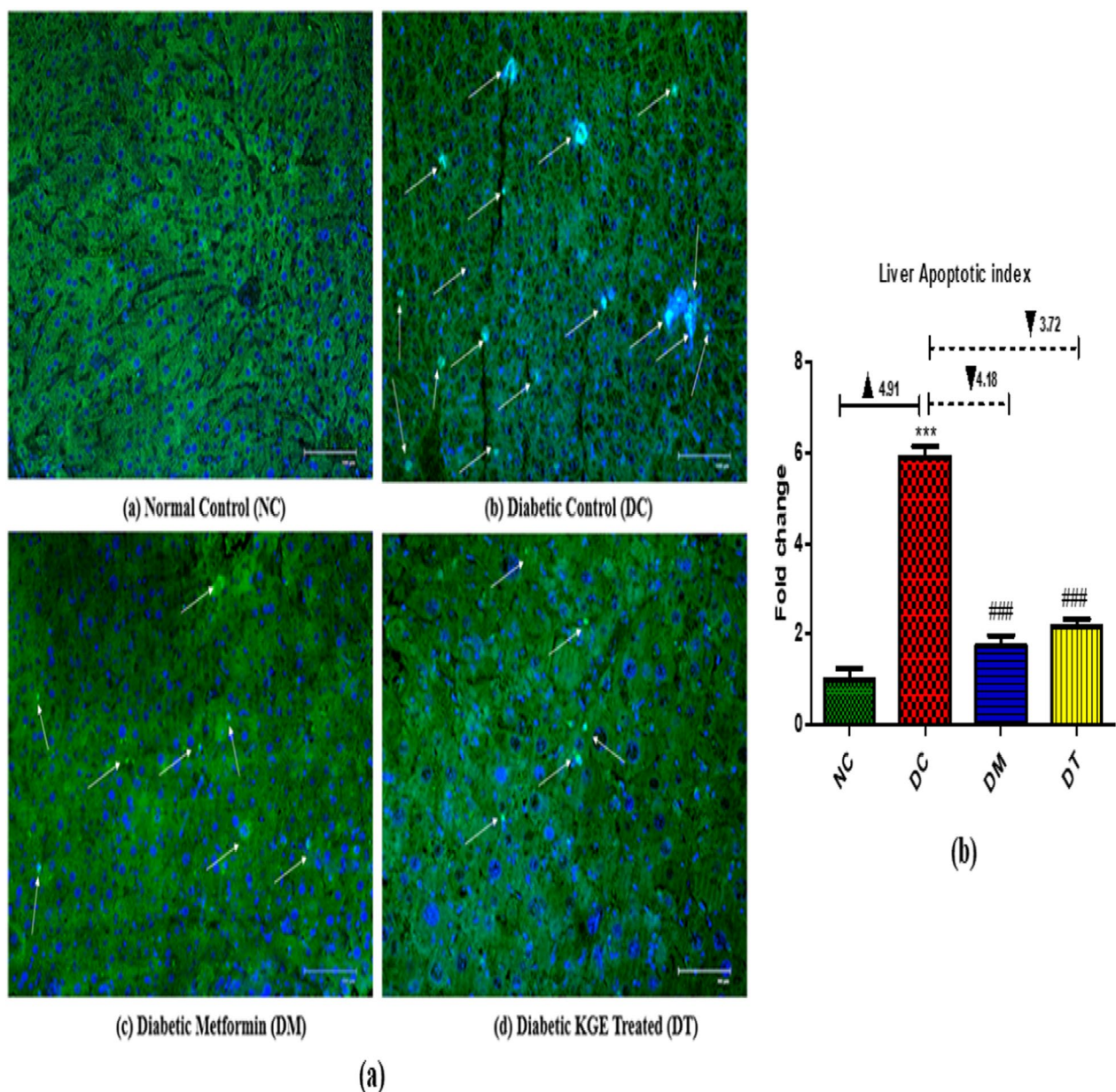


Fig. 11 **a** TUNEL assay of the liver in NC, DC, DM, and DT mice groups. **b** Apoptotic index. Values are expressed as the mean $\pm$ SEM; $n = 3$. Significance difference: *** $p < 0.001$ of DC against the NC group; ### $p < 0.001$ of DM and DT against the DC group

of apoptosis through the regulation and maintenance of glucose homeostasis.

TEM is often used to study organelle ultrastructure. TEM uses several lenses to magnify electrons through a very thin sample to create high-resolution images [43]. TEM has been used to study organelle ultrastructure in a variety of healthy and diseased cells and tissues, thus is very significant in revealing cell function and disease onset [44]. In our study, TEM analysis shows that in the diabetic mice, the hepatocytes had a distorted nucleus

morphology. However, upon supplementation of the diabetic mice with herbal KGE, the hepatocyte morphology was restored, which was comparable to ascorbic and metformin administration. This result further reveals that herbal KGE supplementation has a protective effect on the tissues of STZ-induced diabetic mice.

Insulin is a peptide hormone produced by the β cells of the pancreas. It is essential for controlling how glucose is metabolized. By improving the uptake and metabolism of glucose by cells and promoting its storage in the

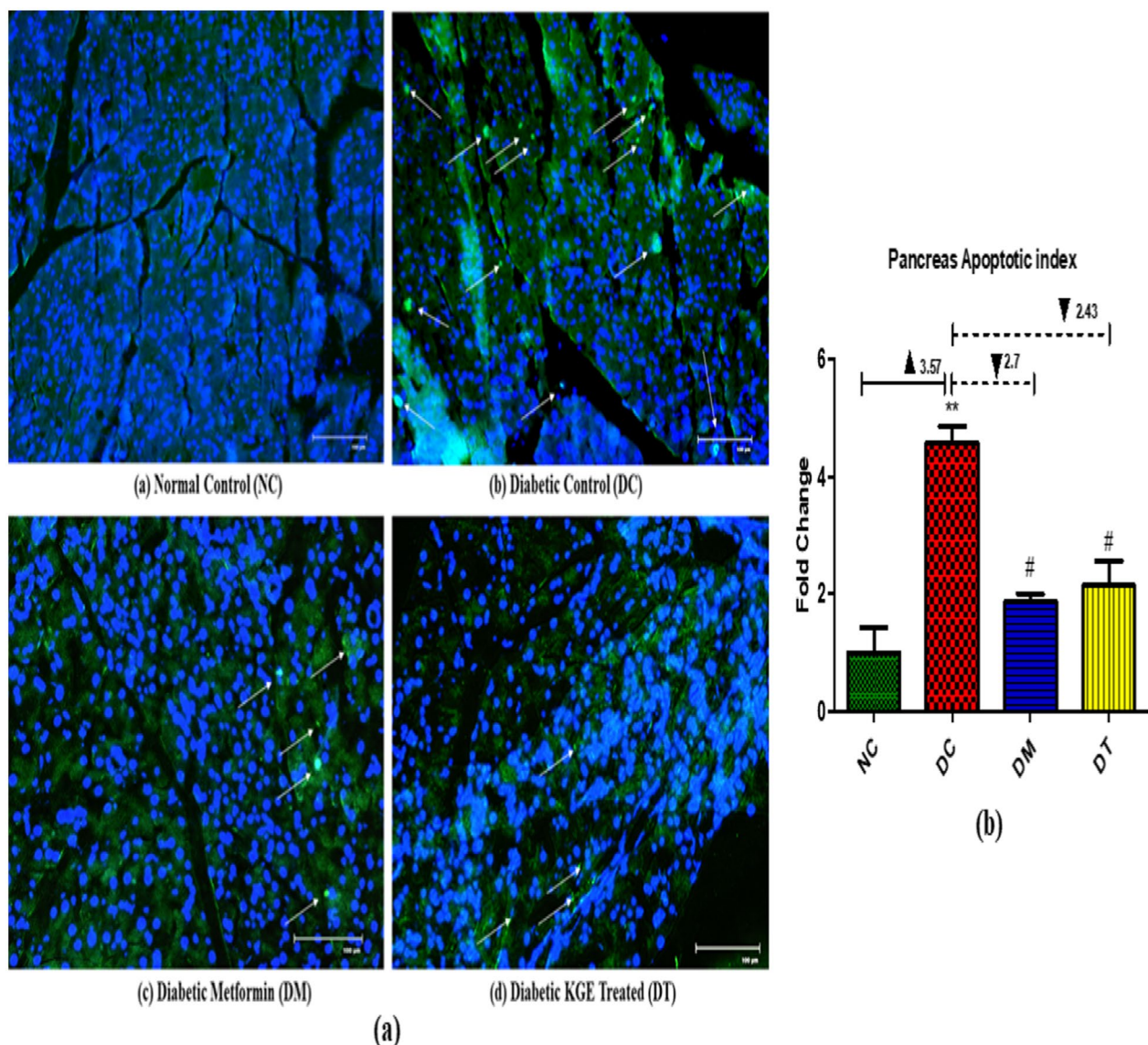


Fig. 12 **a** TUNEL assay of the pancreas in NC, DC, DM, and DT mice groups. **b** Apoptotic index. Values are expressed as the mean $\pm$ SEM; $n = 3$. Significance difference: ** $p < 0.01$ of DC against the NC group; # $p < 0.05$ of DM and DT against the DC group

muscles and liver, insulin lowers blood glucose levels. Additionally, it promotes the synthesis of fats and proteins while preventing their breakdown, thus preserving the body's natural distribution of nutrients [45]. The onset of hyperglycemia and its associated complications can be caused by either absolute or relative insulin secretion as well as defects in its actions. One of the main consequences of numerous studies employing the STZ-induced diabetic mice model is insulin insufficiency due to the pancreatic β cells' rapid deterioration [46]. So, in our study, we have performed the ELISA insulin assay to check the insulin level in the serum and pancreas tissue

of various experimental groups. Our study's results demonstrate that, compared to the normal mice group, the insulin levels in the serum and pancreas of the diabetic control mice group were significantly decreased. On the contrary, in the diabetic mice group treated with standard metformin and glipizide drugs, the insulin level in the pancreas and serum was maintained significantly. Similarly, in the diabetic mice group supplemented with herbal KGE, the insulin level in the pancreas and serum was maintained significantly. This positive effect could result from either the protective effect of herbal KGE in preventing pancreatic damage or from herbal KGE aiding

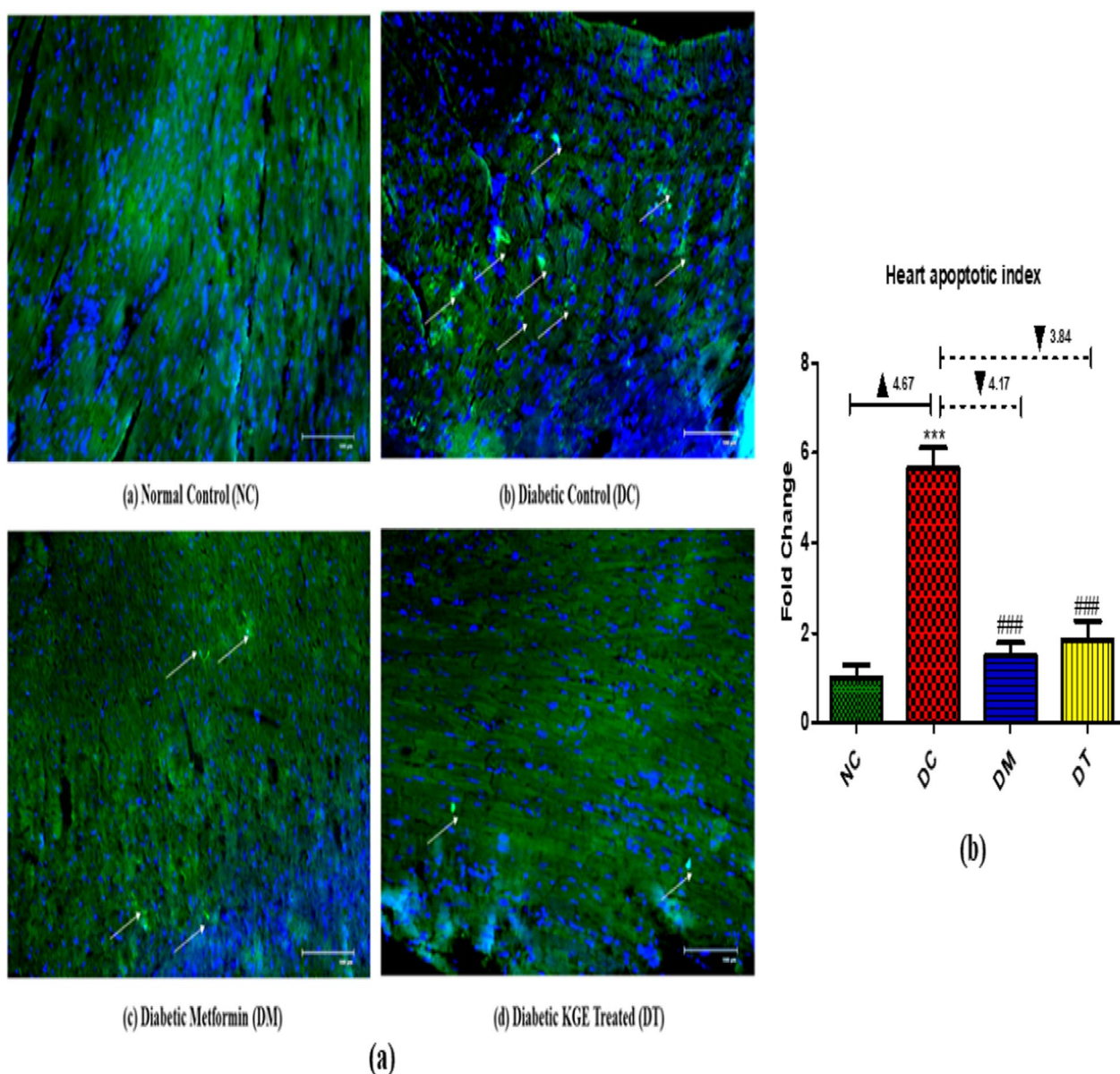


Fig. 13 **a** TUNEL assay of the heart in NC, DC, DM, and DT mice groups. **b** Apoptotic index. Values are expressed as the mean $\pm$ SEM; $n = 3$. Significance difference: *** $p < 0.001$ of DC against the NC group; ### $p < 0.001$ of DM and DT against the DC group

in the regeneration of damaged pancreatic β cells. This was confirmed by the pancreatic histopathology study that has been done in our previous study whereby normal physiology and morphology of the pancreas were restored in the diabetic mice supplemented with herbal KGE [17]. This ultimately provides evidence that herbal KGE provides tissue-protective effect by preventing hyperglycemia and hence decreases oxidative stress and accelerated apoptosis in the process.

Traditional medicines contain bioactive compounds which can be utilized for various therapeutic purposes.

Establishing their molecular targets can aid in the development of novel treatments tailored to individual diseases. In recent years, the binding affinity of potent target drugs to receptor proteins can be predicted computationally using a process known as molecular docking which has grown into a powerful tool for drug development [47]. Thus, with the help of this method, the researcher can identify and target important compounds and predict their therapeutic activities, albeit further validation in *in vivo* studies is needed. GCMS analysis in our previous study shows that EPMC is the bioactive compound that

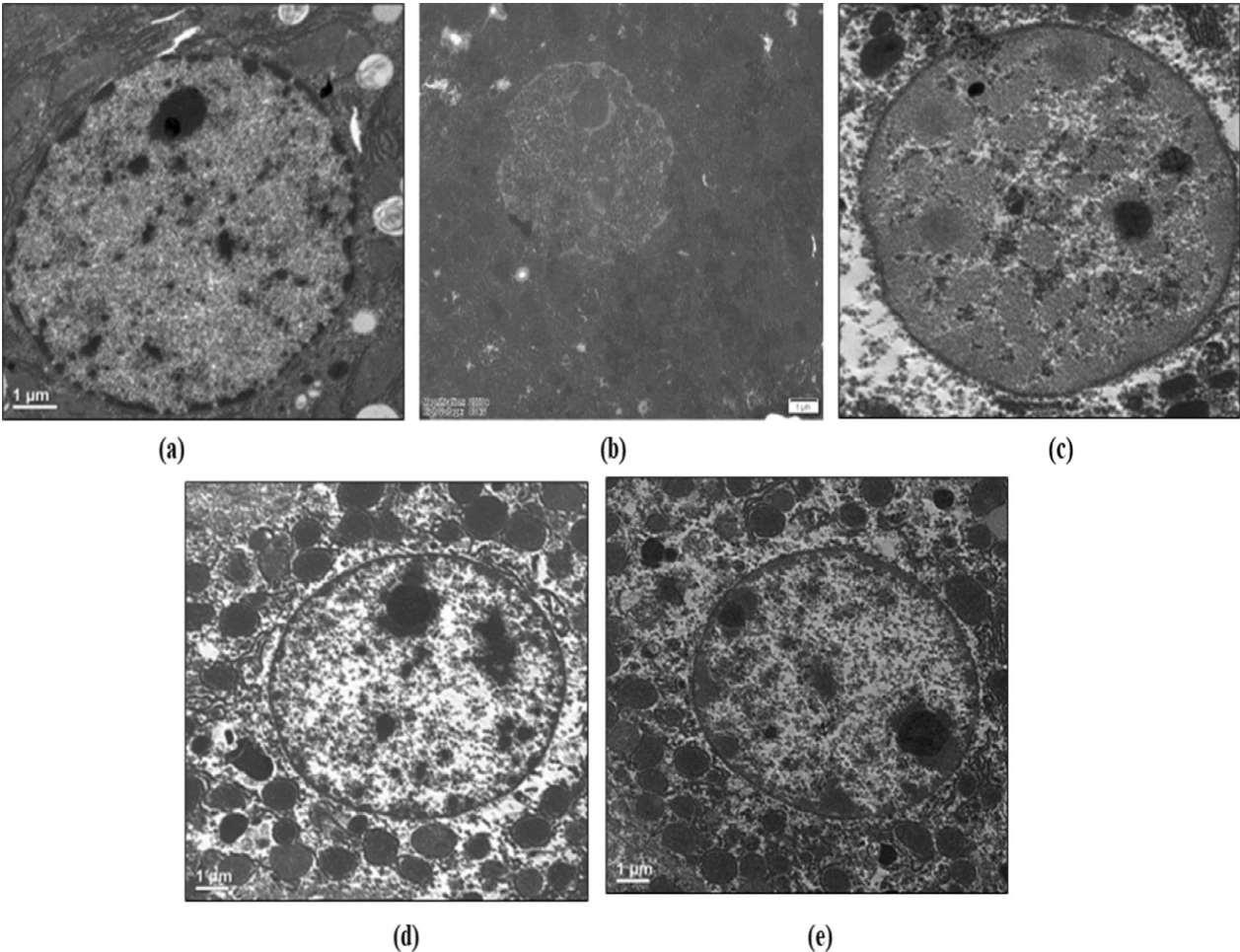


Fig. 14 TEM analysis of the liver in various experimental groups **a** NC, **b** DC, **c** DM, **d** DA, **e** DT

Table 3 Binding affinity and Ligand–protein interactions of EPMC and metformin with CAS-3, BAX, BCL-2

Protein–ligand	Binding affinity (kcal/mol)	Types of interaction	Interacting amino acids and number of bonds or interactions
BAX- Metformin	−4.0	Hydrogen bonds Salt bridges	Thr22 (1), Ser55 (2), Thr56 (2) Asp53 (2)
BAX- EPMC	−4.9	Hydrogen bonds Hydrophobic interactions	Thr22 (1) Leu59 (1), Trp158 (1), Asp159 (1), Leu162 (1)
BCL-2-Metformin	−4.0	Hydrogen bonds Salt bridges	Arg26 (1), Ser75 (2) Glu119 (1)
BCL-2-EPMC	−5.4	Hydrophobic interactions Salt bridges	Asp61(1), Phe71 (1), Val115 (1) Arg68 (1)
CAS-3-Metformin	−3.7	Hydrogen bonds	Lys137 (1), Thr140 (2)
CAS-3-EPMC	−4.5	Hydrogen bonds Hydrophobic interactions Salt bridges	Leu137 (1) Leu136 (2), Lys137 (1), Thr140 (1), Ile160 (1) Arg164 (1)

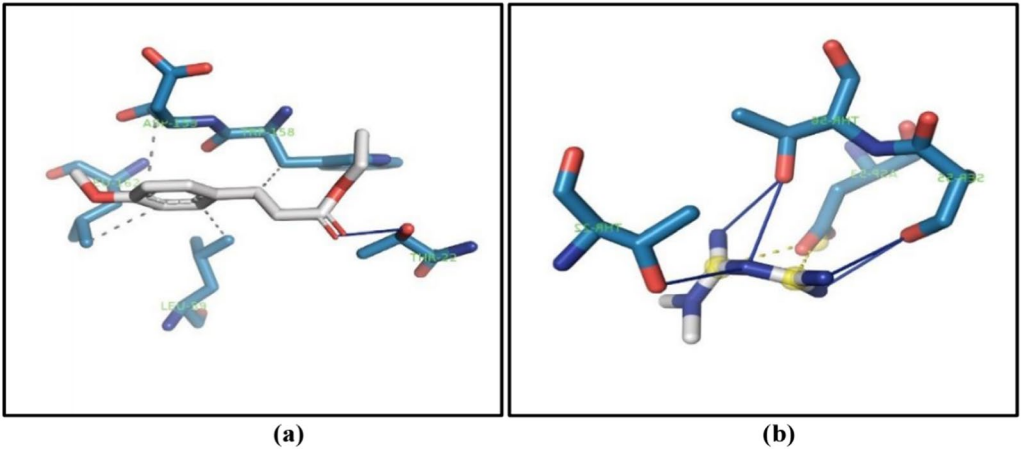


Fig. 15 Docking with BAX (a) EPMC (b) Metformin

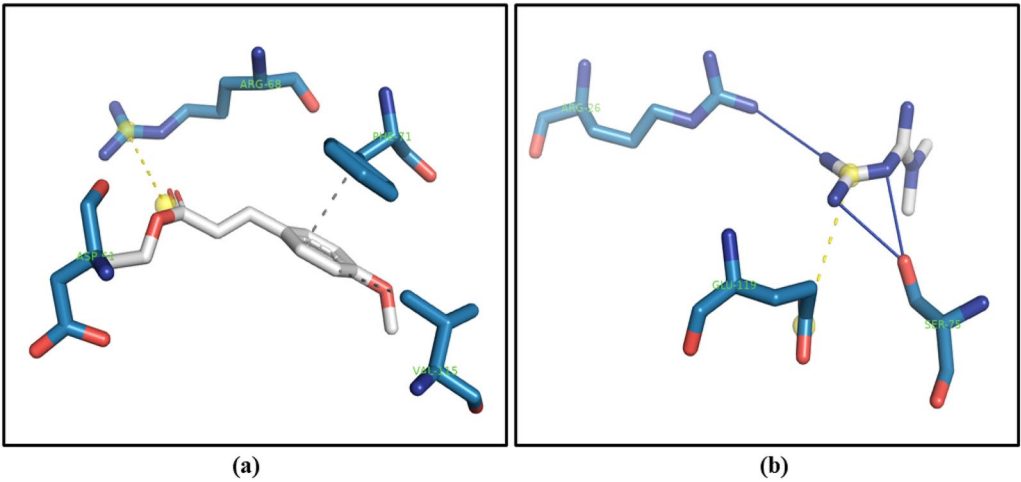


Fig. 16 Docking with BCL-2 (a) EPMC (b) Metformin

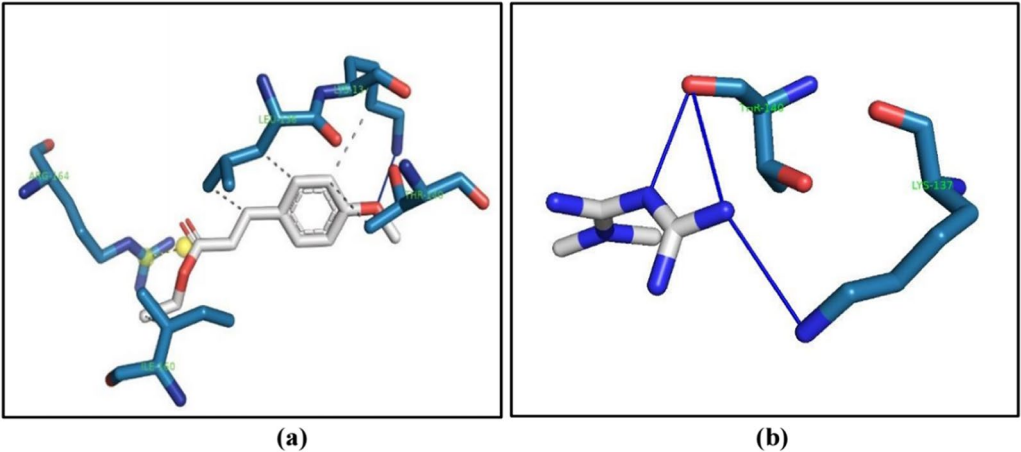


Fig. 17 Docking with CAS-3 (a) EPMC (b) Metformin

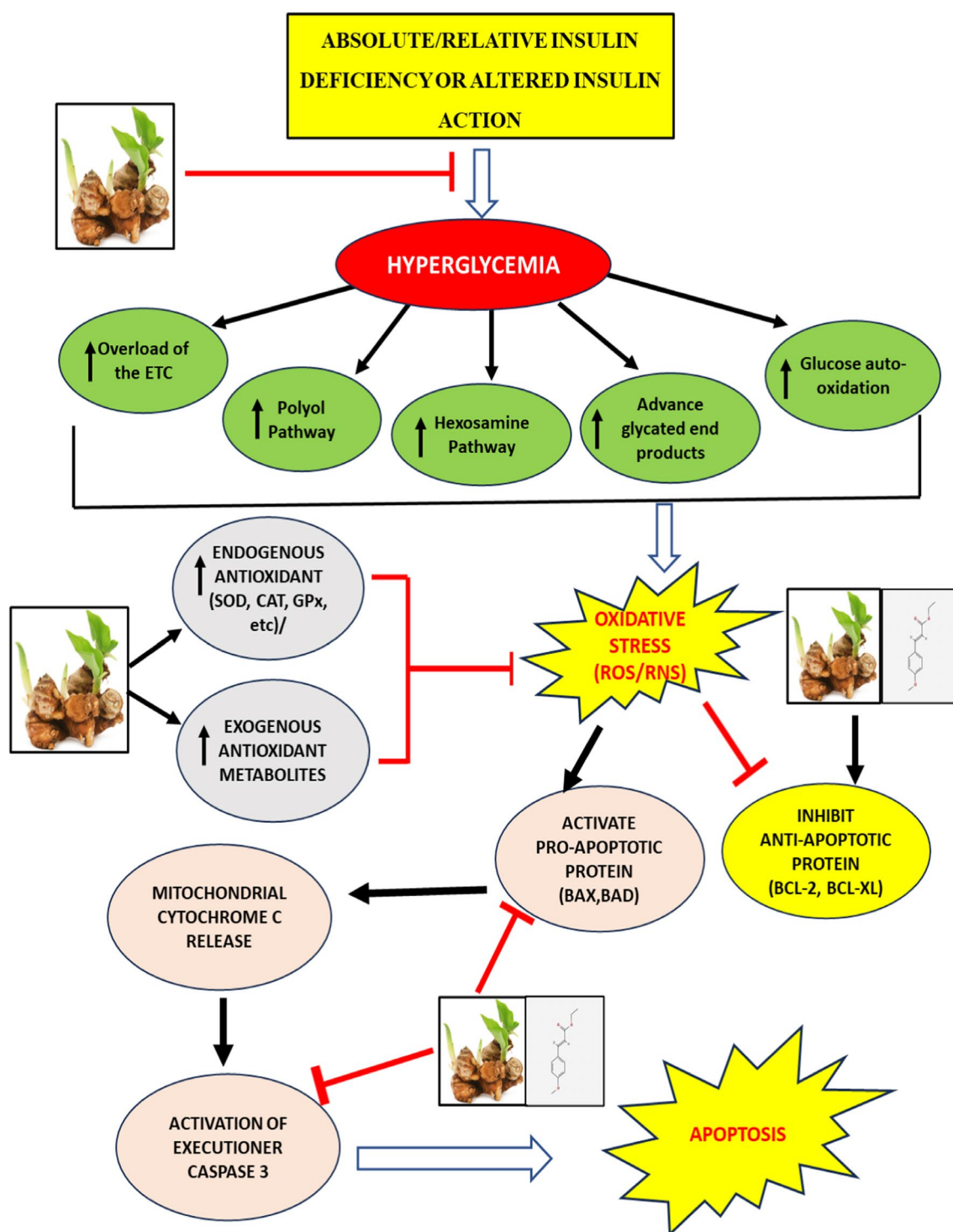


Fig. 18 Proposed mechanism of action of herbal KGE on hyperglycemia-induced oxidative stress and apoptosis

is present in abundant in the herbal KGE [16]. Moreover, HPTLC analysis in the current study confirms the presence of EPMC in the herbal KGE as the bioactive component. Therefore, in this study, docking of EPMC and

standard metformin drug with various proteins involved in the regulation of apoptosis to check its binding affinity was done. Both metformin and EPMC form polar contacts such as hydrogen bonds and salt bridges with

proteins, which is strong; in some cases, additional contacts and interactions for EPMC are seen, which depicts a stronger binding affinity of EPMC than metformin with the proteins. Overall, the binding energy calculation of EPMC is much lesser than metformin for all the apoptotic proteins and, therefore, can bind and modulate them much more efficiently (activation or inhibition). Thus, with this preliminary data, we have concluded that EPMC might have played an important role in alleviating hyperglycemia-induced apoptosis in diabetic mice and further investigations will be performed in our next work to validate this data.

Conclusion

In conclusion, these results suggest that herbal KGE supplementation in STZ-induced diabetic mice improved the altered redox homeostasis by upregulating the activity and expression of endogenous antioxidant enzyme systems as well as providing increased exogenous antioxidant capacity due to the presence of various antioxidant compounds. Furthermore, herbal KGE provides a tissue-protective effect by reducing oxidative stress. Also, herbal KGE supplementation alleviates hyperglycemia-induced apoptosis by increasing the expression of anti-apoptotic protein and simultaneously downregulating the expression of pro-apoptotic thereby inactivating the caspase signaling cascade; ultimately preventing DNA fragmentation and cell death. Herbal KGE supplementation in diabetic mice with herbal KGE prevents a significant decrease in serum or pancreatic insulin levels, indicating herbal KGE's protective ability to avert pancreatic damage or its ability to promote the regeneration of damaged pancreatic β cells. Electron microscopy indicates the protective effect of herbal KGE supplementation on tissue morphology. *In-silico* studies reveal that the bio-active compound EPMC shows good binding affinity to the apoptotic-related proteins and thus might play a role in modulating their activity for the better. Thus, herbal KGE supplementation alleviates hyperglycemia-induced oxidative stress and apoptosis in diabetic mice and can provide a suitable and potent adjuvant in the treatment of diabetes.

Abbreviations

KG	<i>Kaempferia galanga</i>
KGA	KG aqueous extract
KGE	KG ethanolic extract
KGM	KG methanolic extract
KGC	KG chloroform extract
HPTLC	High-performance thin layer chromatography phytochemical fingerprinting
TLC	Thin layer chromatography
EPMC	Ethyl-p methoxy cinnamate
STZ	Streptozotocin
SOD	Superoxide dismutase
CAT	Catalase
ROS	Reactive oxygen species

RNS	Reactive nitrogen species
ETC	Mitochondrial electron transport chain
FRAP	Ferric reducing antioxidant properties
TAC	Total antioxidant capacity assay
DPPH	2,2-Diphenyl-1-picrylhydrazyl
NC	Normal mice
DC	Diabetic mice
DA	Diabetic mice ascorbic acid treated
DM	Diabetic mice metformin treated
DG	Diabetic mice glipizide treated
DT	Diabetic mice herbal KGE treated
RT-PCR	Real-time polymerase chain reaction
qPCR	Quantitative polymerase chain reaction
PBST	Phosphate buffer saline-Tween 20
PBS	Phosphate buffer saline
TUNEL	Terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick-end labeling
TdT	Terminal deoxynucleotidyl transferase
INS	Insulin
TEM	Transmission electron microscopy studies
Protein Data Bank, Quaternary	PDBQT
ELISA	Enzyme linked immunosorbent assay

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s43094-025-00772-z>.

Supplementary material 1

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Author contributions

FPN contributed to conception and design of the work, collection, and preparation of sample (in vitro, in vivo, and in-silico studies), analysis, and interpretation of data, and manuscript preparation. RS contributed to resources, in vivo studies, and manuscript preparation. DR contributed to resources, in-silico studies, and manuscript preparation. SB contributed to conception and design of the work, manuscript review, and guidance.

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Availability of data and materials

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Declarations

Ethics approval and consent to participate

Yes, the Institutional Animal Ethics Committee approved with ethics certificate IEC/MS/Misc/19 (BALB/c Swiss albino mice).

Consent for publication

Not applicable.

Conflict of interests

No conflict of interest.

Studies involving plants

The rhizome of *K. galanga* was collected from the forest of Nongpoh, Ri-Bhoi district of Meghalaya, India, and was authenticated by Dr. C. Deori, Scientist-in

Charge, Botanical Survey of India (BSI), Eastern Regional Centre having the authentication no BSI/ERC/Tech/2019–20/655.

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