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Research Article

Salutary Effect of *Commelina benghalensis (Linn.)* Aerial Parts on Streptozotocin Induced Diabetes Linked Complications in Rat: Effective Extract Selection Study

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ABSTRACT

The experiment was designed to identify the most effective extract of *Commelina benghalensis Linn.* aerial parts for the rectification of carbohydrate metabolic disorder, oxidative stress and hyperlipidemia in diabetic rats, developed with a single intramuscular injection of streptozotocin (4 mg/0.1 M citrate buffer/0.1 kg of body weight). Diabetic rats were treated with 0.02 g of hydro-methanol (60:40) or hydro-ethanol (60:40) or aqueous extract orally per 0.1 kg body weight in 0.5 mL distilled water for 28 days and sacrificed on the 29th day. Glycemic sensor levels, carbohydrate metabolizing enzyme activities, lipid profile, oxidative stress, toxicity markers, and pancreatic histological changes were assessed using standard protocols. Treatment with the mentioned extracts of *C. benghalensis Linn* significantly (p <0.05) improved body weight, fasting blood glucose, serum insulin, lipid profile, and carbohydrate metabolic enzyme kinetics in the liver, kidney, and skeletal muscle in treated groups compared to untreated diabetic control group. Additionally, treatment of said extracts of *C. benghalensis Linn* significantly restored (p <0.05) the levels of oxidative stress sensors and pancreatic histology as untreated control group. Among the extracts, the hydro-ethanolic extract showed the highest efficacy for rectifying glycemic status, lipid profile, and oxidative stress condition for developing a safe, affordable, and multi-targeted antidiabetic herbal drug.

Introduction

Diabetes mellitus has emerged as a significant global health crisis, currently the 4th leading cause of death, followed by cardiovascular disease, cancer and respiratory disease, contributing to the substantial mortality burden worldwide.[1] It is a lifestyle disease that is strongly associated with unhealthy food style. Due to its complex and multifaceted nature, it is referred to now as a syndrome. The pathophysiology of diabetes is primarily driven by pancreatic beta-cell dysfunction, which results in inadequate insulin secretion. This dysfunction leads to chronic hyperglycemia, a hallmark of this disease.[2] Worldwide, both in industrialized and developing nations, the prevalence of diabetes is a significant concern. Currently, 537 million individuals are affected globally by this metabolic disorder. However, estimates suggest that this figure is expected to rise significantly by the year 2045, exceeding approximately 700 million individuals affected by diabetes.[3] In hyperglycaemic state, there is an alteration in enzymes involved in the carbohydrate metabolic pathway, resulting in the accumulation of metabolic intermediates and disrupting normal insulin signaling.[4] Increased glycolytic flux leads to elevated reactive oxygen species (ROS) production through interruption of the electron transport chain (ETC), glucose auto-oxidation, increased advanced glycated end-product formation and the activation of the polyol pathway.[5] These increased levels of reactive oxidants

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(singlet oxygen, hydrogen peroxide, nitric oxide, hydroxyl radical, etc.) alter antioxidant defenses through the numerous interconnected enzymatic, non-enzymatic, and mitochondrial processes. This interconnected process leads to cellular damage, causing beta cell dysfunction, resulting in impairment in insulin signaling and reduced glucose utilization.[6] Consequently, it has a prominent impact on various body systems, leading to both macro and microvascular complications.[7] Insulin therapy remains indispensable for type 1 diabetes (T1D) management and oral anti-hyperglycaemic medications play a crucial role in type 2 diabetes (T2D) treatment for promoting glucose utilization at a cellular level, but the long-term use of these medicines is associated with so many side effects.[8] Ayurveda, the traditional Indian medical system, has already provided a promising avenue for the treatment of diabetes. It is difficult to bridge the gap between the ancient knowledge of ayurveda and contemporary biological understanding. The "bedside to bench" method, another translational approach that involves research at the cellular and molecular levels, represents a viable avenue despite its complexity.[9] Through the integration of contemporary scientific knowledge with the inputs from ayurveda, a novel approach has been adopted for diabetes management, ultimately improving the lives of millions.[10,11] *Commelina benghalensis* (*Linn.*) is one type of perennial herb that belongs to the Commelinaceae family, locally known as "Kanshira," available throughout India. Kanshira is a leafy vegetable that has various pharmacotherapeutic effects such as infertility management**,** anti-inflammatory activity**,** antimicrobial properties, oxidant scavenging potentiality**,** hepatoprotective, and anticancer activity.[12] Although the literature review concentrated on the plant's folk reputation for antidiabetic properties by addressing only blood glucose level (BGL) measuring and phytochemical(s) analysis to establish the antidiabetic activity of this plant, BGL assessment is not sufficient enough to fulfill plants' antidiabetic potential assessment, which is the scientific gap of previous research.[13] Extensive scientific investigation beyond BGL is needed to unlock the mode of action regarding the antidiabetic efficacy of aerial parts of the plant. This includes the assessment of serum insulin, carbohydrate metabolic and antioxidative enzyme activities, lipid profile, histological analysis, and toxicity sensors. This assessment helps to know the overall mechanism underlying plant-derived glucose metabolism and utilization in cells, oxidative balance, and safety, aiding to alleviate diabetes-associated co-morbidity, the novelty of this work. The current study highlights the selection of potent extract using different solvents such as hydro-methanol extract (HMECB), hydro-ethanol extract (HEECB), and aqueous extract (AQECB) of *C. benghalensis (Linn.)* aerial parts' for correcting diabetes and oxidative stress associated morbidity in experimental diabetic model rats. Based on the outcome of the literature review and the pilot study in our laboratory, it is hypothesized that the effective phytomolecule(s) present in most potential extracts may rectify diabetic complications by managing oxidative stress and glucose utilization at a cellular level. For testing this hypothesis, the experiment has been designed to find out the most promising extract for this purpose which is also safe and multi-targeted for the comprehensive management of diabetes mellitus.

Materials And Methods

Collection of Plant Material

The aerial parts of *C. benghalensis* (*Linn.*) were collected from the Purba Medinipur local field during the monsoon season at the end of July 2022. This plant is a climate-dependent, perennial or annual herb. Authentication number of the plant was received from the Botany and Forestry Department, Vidyasagar University (Herbarium specimen no. *C. benghalensis*/VU/Bio/09/22).

Extraction

The dried plant materials were powdered and extracted with different solvents. The menstruum was prepared separately using hydro-methanol (3:2), or hydro-ethanol (3:2), or aqueous solvent. About 50 g of powder was suspended in 1000 mL of double distilled water or hydro-methanol (3:2), or hydro-ethanol (3:2) solution. The above three mixtures (menstruum) were allowed to macerate for 48 hours at a controlled temperature (25 ± 2°C) with intermittent stirring every 2 hours intervals. The hydro-methanol, hydro-ethanol, or aqueous filtrated extract were subjected to reduced pressure (40–60°C) under a rotary evaporator for the efficient evaporation of the solvent. Finally, condensed residual plant material was collected, dried, and stored in an air-proof glass container in a refrigerator (2–8°C) for use in experimental work.[14]

Chemicals

Streptozotocin, nicotinamide adenine dinucleotide phosphate (NADP), adenosine triphosphate (ATP), thiobarbituric acid (TBA), trichloroacetic acid (TCA), HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) and additional chemicals were procured from Sigma Aldrich Diagnostic Ltd, Maharashtra, India. All reagents utilized in this investigation were in analytical graded. Meril Diagnostic Ltd, India, provided the kit for toxicity sensor assessment. Kits from Span Diagnostic, Surat, India, were used for lipid profile assessment.

Animals

Thirty healthy in-breed Wistar strain male albino rats, weighing about 120 ± 10 g, 2 months old, were purchased from Saha enterprise. Keeping rats in a controlled environment with 12 hours circadian cycle of day and night for 15 days before their use in an experiment. This acclimatization period allows the animals to adjust

physiologically and behaviorally, reducing stress-related variables that could confound experimental results. The animals have been separated into five groups, each containing six animals.

Ethical Statement

Animal care and maintenance were done as per the direction given by the Institutional Animal Ethics Committee (IAEC) and the approval no. was VU/IAEC/CPCSEA/7/7/2022, dt-22/11/2022. The experiment was conducted in accordance with the guidance of the Committee for Control and Supervision of Experiments on Animals (CCSEA), Govt. of. India.

Diabetes Induction

Streptozotocin (STZ) was injected intramuscularly at a single dose of 40 mg/kg of body weight for inducing diabetes in a rat model. To equalize the degree of physical stress experienced by the diabetes group in respect to the injection, an intramuscular injection of citric acid buffer with pH 4.5 at 1-mL/kg dose was delivered to the control group. Blood glucose levels were recorded on the eighth day after the STZ injection following a 12-hour fast. Animals with fasting blood glucose (FBG) levels between 300 to 350 mg/dl were considered diabetic model animals for this investigation.[15]

Study Design

Thirty rats were grouped into 5 different categories and each category has six rats.

Group I- untreated control group (UCG)

Healthy normoglycemic rats having FBG levels 70 to 80 mg/dl received orally 0.5 mL distilled water (DW) /0.1 kg of BW using gavage daily at 9 AM on an empty stomach.

Group II- untreated diabetic control group (UDCG)

Hyperglycaemic rats (300–350 mg/dl) received DW orally at 0.5 mL/0.1 kg of body weight (BW) as the previous group.

Group III- HMECB treated group

The HMECB was administered orally through gavage to diabetic rats in this group at a dose of 0.02 g/0.5 mL DW/0.1kg BW one time a day.

Group IV-HEECB treated group

As previously mentioned, HEECB was given orally to each diabetic rat in this group at a dosage of 0.02 g diluted in 0.5 mL distilled water per 0.1 kg of BW.

Group V- AQECB treated group

Each diabetic rat of this group received a 0.02 g oral dose of AQECB, which was dissolved in 0.5 mL DW /0.1 kg BW as before.

The treatment was continued for four weeks. On the 29th day of treatment, following a 12 hours food deprivation state, all rats were sacrificed under controlled ether anesthesia to minimize the degree of distress and discomfort. Blood samples were drawn from the dorsal aorta using a 3 mL syringe and FBG level was recorded. After 45 minutes, serum separation was completed for concerning tests. All necessary vital organs, including the kidney, liver and skeletal muscle (SM) were separated, washed in 0.9% of physiological saline, socked in filter paper, and stored at -20°C before being utilized in an enzymatic assay. The dissected pancreas was processed for histological study.

Body Weight Measurement

The body weights (BW) of all rats were measured before starting of treatment regimen and after completion of the treatment duration using the weighing machine.

Fasting Blood Glucose and Insulin Level Determination

Throughout the course of the next 28 days of the experiment, the FBG levels of UCG, UDCG, and extract-treated rats were monitored using a glucometer. The value was expressed as mg/dl.[15] Serum insulin level was checked by rat insulin Ray Bio (USA) ELISA kit on the 29th day of treatment. The value was indicated in terms of µIU/mL. [16]

Evaluation of Carbohydrate Metabolic Sensors

Hexokinase (HEX) activity was studied in the liver, kidney and SM using the standard protocol and absorbance was recorded at 340 nm. The enzyme activity was expressed as µg/mg of tissue.[15] Glucose-6-phosphatase (G6Pase) kinetics was measured using published protocol. Absorbance was noted at 340 nm. The enzyme catalytic activity was measured based on the mg of inorganic phosphate liberated per g of tissue.[15]

Glycogen

Glycogen level in the liver, kidney and SM was quantified biochemically as per the published protocol.[17]

Lipid profile

Measurement of total cholesterol (TC), triglyceride (TG), high-density lipoprotein-cholesterol (HDL-C), and low-density lipoprotein-cholesterol (LDL-C) levels in serum were conducted using the reagents supplied in the kit. (Meril Diagnostics Ltd, India).[18]

Oxidative stress marker assay in metabolic tissue

Separate homogenization of renal and hepatic tissues was done [50 mg/mL, 0.05 (M) isotonic phosphate buffer solution, pH -7.4] and centrifuged to collect the supernatant. Catalase activity was measured at spectrophotometric cuvette after combining 100 µL of tissue supernatant with 500 µL of 0.00035M H_2O_2 and 2500 µL of ion-free water mixture. The sample's absorbance was recorded at 240 nm

at 30 seconds intervals for 180 seconds. The result was presented in terms of mM of H_2O_2 consumption per milligram of tissue per min (mm H_2O_2/mg tissue/min).[19] For evaluating SOD kinetics, 2.0 mLof 0.05M Tris buffer, pyrogallol (20 µL), and tissue supernatant (20 µL) were taken. The absorbance of the sample was recorded at 420 nm. The enzyme turnover rate was indicated as unit/mg of tissue/min.[19] The amount of lipid peroxidation in the liver was evaluated using the malondialdehyde (MDA) content, following a previously described method.[19]

Acute Toxicity Study

The behavioral as well as symptomatic changes of the experimental rats were constantly noticed for 2 days after the administration of different solvent extract of *C. benghalensis* (*Linn.*) (DSECB) aerial parts. Additionally, they were monitored for immediate mortality within 72 hours of extract post-administration. This comprehensive observation period allowed for a detailed assessment of the acute toxicity of the DSECB.[14]

Chronic Toxicity Study

Assessment of serum urea, uric acid, creatinine, acid phosphatase (ACP), and alkaline phosphatase (ALP)

Serum urea, uric acid, and creatinine levels, along with serum ACP and ALP activities, were measured by using standard kits (Meril Diagnostics Ltd, India) with optical density assessment for analysis.[18]

Phytomolecule analysis of DSECB

Qualitative screening of phytomolecule(s) was analyzed from the DSECB to identify the nature of phytocompound(s) present in plant extracts.[20]

Histological study

From every group, the pancreas was dissected and fixed in a picric acid-formaldehyde-acetic acid-based solution. After 24 hours duration, said tissue was dehydrated in graded ethanol (50, 70, 80 90%, absolute alcohol) and xylene was used to clear the ethanol from the tissue before embedding it into the paraffin wax. Finally, the tissue was embedded in the paraffin wax for the structural integrity of preserved tissue and very thin sections (5 µm) were prepared using Leica microtome. Hematoxylin and eosin were used for the staining of the staining the deparaffinized tissue sections. Using a computer-aided microphotography system, histological analysis was performed on stained sections.[21]

Statistical Analysis

The acquired data was statistically overviewed by calculating the mean and standard error. Analysis of variance (ANOVA) was determined to assess the significance at the level of p <0.05 followed by multiple comparisons using the Student's one-tail 't' test.[22]

Results

Body Weight (BW)

A significant reduction in BW was observed in the UDCG compared to UCG. The finding also indicated the remarkable BW improvement (p <0.05) in DSECB-treated groups against UDCG. Among the treated groups, the HEECB extract-treated group showed the most significant recovery (p < 0.05) (Table 1).

Glycemic Sensors

Fasting blood glucose and serum insulin levels measurement and carbohydrate metabolic sensors assessment

A remarked increase in FBG, along with a decrease in serum insulin level, were observed in UDCG compared to UCG (p <0.05). After 4 weeks of DSECB treatment, a remarkable improvement (p <0.05) was noticed in FBG and insulin levels. Recovery percentages for FBG in HMECB, HEECB, and AQECB treated groups were 40.81, 46.65, and 44.18%, respectively, while for insulin, the recovery percentages were 45.52, 70.40, and 19.89%. Simultaneously, reduced HEX activity and glycogen level, with elevated G6Pase activity in hepatic, renal, and SM tissues of UDCG, were remarkably corrected (p <0.05) toward UCG levels after 4 weeks of uninterrupted DSECB treatment. Recovery percentages for HEX in liver, kidney, and SM tissues were 50.08, 45.35, and 46.79% in HMECB treated group; 58.05, 56.24, and 54.94% in HEECB treated group, and 46.4, 34.67, and 39.11% in AQECB treated group, respectively. The recovery percentages for G6Pase in the above three tissues were 23.41, 20.63, and 20.27% in HMECB treated group, 35.96, 32.53, and 31.99% in HEECB treated group, and 13.9, 10.48, and 28.23% in AQECB treated group. Glycogen recovery values were 45.45, 41.78, and 47.86% in HMECB treated group; 68.18, 58.27, and 62.63% in HEECB treated group; and 21.95, 18.47, and 26.98% in AQECB treated

Table 1: Remedial effect of DSECB on BW

Groups	*Initial BW (g)*	*Final BW (g)*
UCG	123.01 ± 1.42^a	151.28 ± 1.80^a
UDCG	120.49 ± 2.12^a	108.94 ± 3.73^b
HMECB	121.75 ± 0.57^a	139.49 ± 3.02^c (28.04% against UDCG) ↑
HEECB	122.48 ± 1.55^a	144.12 ± 2.59^c (32.29% against UDCG) ↑
AQECB	124.34 ± 1.74^a	132.45 ± 1.57^c (21.58% against UDCG) ↑

The obtained values of six samples were represented as Mean ± SEM. Variance analysis (ANOVA) was executed followed by 'Multiple comparison student's one-tail t-test.' Values with separate superscripts (a-c) in the vertical column significantly (p <0.05) varied from each other.

Table 2: Corrective effect of DSECB on lipid profile and toxicity markers

Name of test profile		*UCG*	*UDCG*	*HMECB*	*HEECB*	*AQECB*
Lipid profile	TC (mg/dl)	112.68 ± 2.37[a]	164.41 ± 1.61[b]	111.98 ± 1.54[c] (31.88% against UDCG) ↓	106.61 ± 1.44[d] (35.15% against UDCG) ↓	133.30 ± 2.66[e] (18.92% against UDCG) ↓
	TG (mg/dl)	83.95 ± 1.32[a]	137.69 ± 1.35 [b]	115.47 ± 2.08[c] (16.13% against UDCG) ↓	93.67 ± 1.19[d] (31.97% against UDCG) ↓	121.56 ± 0.99[e] (11.71% against UDCG) ↓
	LDL-C (mg/dl)	51.65 ± 0.80[a]	123.24 ± 0.98 [b]	75.40 ± 1.27 [c] (38.81% against UDCG) ↓	62.66 ± 1.11 [d] (49.15% against UDCG) ↓	80.38 ± 0.89 [e] (34.77% against UDCG) ↓
	HDL-C (mg/dl)	38.37 ± 0.89[a]	20 ± 1.06 [b]	28.54 ± 1.62[c] (42.7% against UDCG) ↑	31.09 ± 1.76 [d] (55.45% against UDCG) ↑	27.17 ± 0.82 [e] (35.85% against UDCG) ↑
Chronic toxicity profile	Serum urea mg/dl)	37.84 ± 2.18[a]	65.37 ± 1.20[b]	50.88 ± 1.09[c] (22.16% against UDCG) ↓	44.38 ± 1.47[d] (32.10% against UDCG) ↓	50.93 ± 0.81[c] (22.08% against UDCG) ↓
	Serum uric acid (mg/dl)	2.36 ± 0.11[a]	7.48 ± 0.24[b]	4.84 ± 0.55[c] (35.29% against UDCG) ↓	3.99 ± 0.17[d] (46.65% against UDCG) ↓	4.91 ± 0.51[d] (34.35% against UDCG) ↓
	Serum creatinine (mg/dl)	0.53 ± 0.02[a]	0.84 ± 0.01[b]	0.72 ± 0.02[c] (14.28% against UDCG) ↓	0.61 ± 0.02[d] (27.38% against UDCG) ↓	0.63 ± 0.02[d] (25% against UDCG) ↓
	Serum ACP (Unit/dl)	9.37 ± 0.079[a]	24.15 ± 0.31[b]	16.78 ± 0.11[c] (30.51% against UDCG) ↓	15.66 ± 0.21[c] (35.15% against UDCG) ↓	16.04 ± 0.09[c] (33.58% against UDCG) ↓
	Serum ALP (Unit/dl)	8.35 ± 0.12[a]	17.44 ± 0.08[b]	14.68 ± 0.26[c] (15.82% against UDCG) ↓	13.34 ± 0.15[c] (23.50% against UDCG) ↓	13.81 ± 0.18[c] (20.81% against UDCG) ↓

Mean ± SEM where n = 6 was used for data representation. Variance analysis (ANOVA) was executed, followed by 'Multiple Comparison Student's one-tail t-test'. Tabulated values and their superscripts (a-e) differ significantly (p <0.05) from each other for lipid profiles; in the case of chronic toxicity markers, superscripts (a-d) in the rows significantly (p<0.05) differ from one another.

Table 3: Qualitative cum semi-quantitative analysis of phytomolecules present in DSECB

Phytochemical constituents	*Test name*	*Determinants*	*HMECB*	*HEECB*	*AQECB*
Alkaloid	Dragendroff test	Precipitation (orange-red)	+	++	-
Flavonoids	Ferric chloride test	Blackish red	++	+++	+
Tannin	Ferric chloride test	Brownish green	++	+++	+
Phenol	Ferric chloride test	Bluish black	++	+++	+
Glycosides	Kellar killan test	Browning junction	-	-	-
Steroid	Libermann-Burchard reaction	Greenish black	-	-	-
Terpenoids	Salkowski test	Reddish brown	+	++	-
Anthocyanin	Brontagers test	Pink	-	-	-
Saponin	Foam test	Soapy appearance	-	-	-

'+' indicate present, '-' indicate absence
"++" and "+++" indicate the double and triple intensity of phytomolecule present in plant extract in respect to '+'

group. The HEECB treatment showed the most significant effect among all the groups (Fig. 1A-E).

Oxidative Stress Biomarkers

The reduced antioxidative enzyme kinetics with higher TBARS level in liver and kidney tissue of UDCG was noticeably (p <0.05) improved towards UCG after 4 weeks of administration of DSECB. In the HMECB, HEECB and AQECB treatment groups, the recovery percentages for catalase activity in hepatic tissue were 42.57, 69.8, and 14.35%, whereas in renal tissue, the recovery percentage values was 43.07, 71.28, and 9.74% respectively. For SOD

activity, recovery percentage in liver tissue were 54.54, 74.64, and 27.27%; and in kidney tissue, 55.12, 76.28, and 30.76%, respectively. Furthermore, TBARS level recovery percentages in the liver were 39.43, 44.97, and 35.09%; and in the kidney, 32.38, 42.31, and 20.74%, respectively. Out of all the treatment groups, the HEECB-treated group exhibited the maximum recovery percentage (Fig. 2A-C).

Lipid Profile in Serum

Deviated lipid profile level in serum of UDCG was recovered significantly (p <0.05) towards the UCG following 28 days of continuous treatment with DSECB. The highest level of recovery was observed in the HEECB-treated group among other treated groups (Table 2).

Acute Toxicity Study

After 4 weeks of continuous treatment, no such abnormal behavioral changes, viz backward walking, aggressive behavior, tremors, rage, depression, food and water intake deprivation, dizziness, or no irritation at the time of handling; additionally, symptomatic changes such as hair erection, salivation, postural gait abnormality, lethargy, loose stool, urination frequency, dermatological abnormality were observed in the extract treated groups.

Chronic Toxicity Study

Activities of ACP, ALP and levels of urea, uric acid and creatinine in serum

Toxicity markers such as ACP and ALP activities, serum urea, uric acid and creatinine levels were remarkedly increased in UDCG (p <0.05) compared to UCG. After uninterrupted treatment with DSECB at the indicated dose for 4 weeks, a significant restoration (p <0.05) of such toxicity sensors was observed against UDCG. Among the various treated groups, the HEECB-treated group showed the highest level of recovery percentage (Table 2).

Preliminary Screening of Phytomolecule

The outcome of the qualitative analysis of DSECB showed different types of Phytomolecules, which were summarized in Table 3.

Histoarchitectural Analysis of Pancreas

The UCG (Fig. 3A) showed the normal size of pancreatic islets with dense cell numbers, while in the UDCG (Fig. 3B), pancreatic islets were shrunken with a reduced cell number compared to the UCG. The cell population was improved in the HMECB (Fig. 3C), HEECB (Fig. 3D), and AQECB (Fig. 3E) treated groups relative to the UDCG. The group treated with HEECB showed more significant improvements in pancreatic histology, displaying the control size of islets of Langerhans with more or less similar cell population density. (Fig. 3A-E)

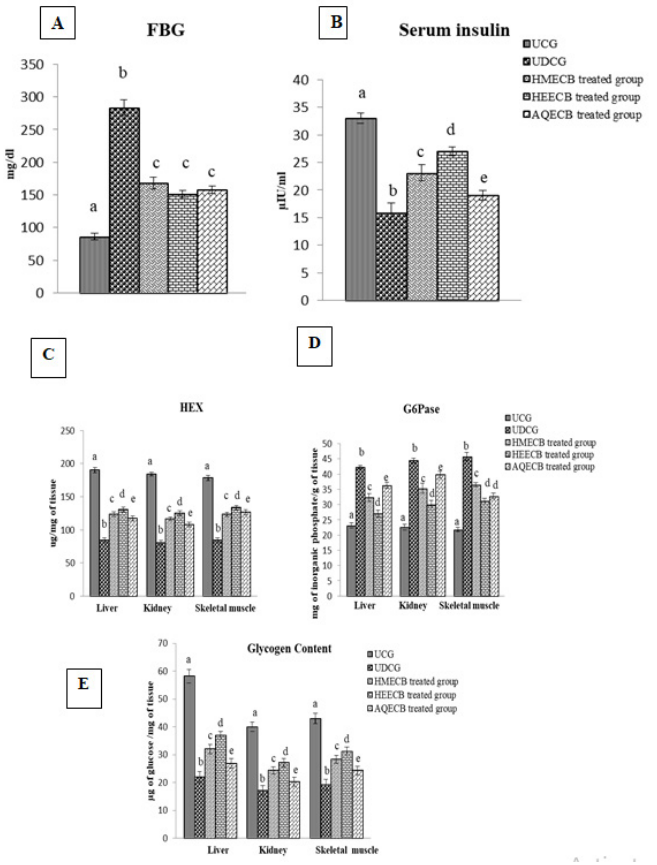

Fig. 1: Improving effect of DSECB on sensitive glycaemic sensors. [A] FBG, [B] serum insulin level, [C] HEX, [D] G6Pase and [E] Glycogen in metabolic tissues. Values obtained from results were represented as Mean ± SEM, where n = 6, Variance analysis (ANOVA) followed by 'Multiple Comparison Student's one-tail t-test'. Bars with separate superscripts (a-e) significantly (p <0.05) differ from each other.

Discussion

The interconnected processes of diabetes, oxidative stress, and hyperlipidemia create a complex network that exacerbates the diabetic condition. Chronic high blood sugar resulting advanced glycation-associated end-products formation, provoking inflammation, and oxidative stress, which cause cellular damage and dysfunction, particularly in beta cells, reducing insulin secretion. Additionally, elevated lipids disrupt insulin signaling, increase oxidative stress and further damage the liver as well as blood vessels. These processes collectively stimulate a cascade of deleterious events that lead to significant tissue damage, including in the pancreas, liver, and vascular system.[23]

Body weight loss in UDCG may be due to insulin deficiency, which leads to poor glycaemic control and increased protein catabolism. In the absence of sufficient glucose for energy, the body utilizes protein from muscle tissue, breaking it down into amino acids for gluconeogenesis. This process results in muscle and weight loss, has

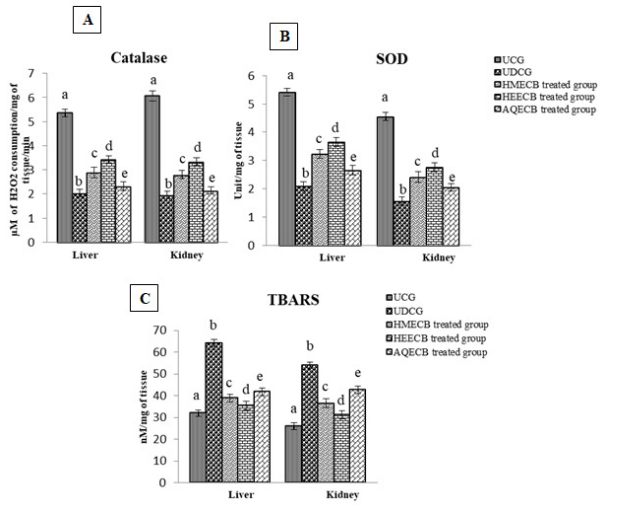

Fig. 2: Ameliorative effect of DSECB on antioxidative sensors and TBARS level in metabolic tissue. Kinetics of [A] Catalase [B] SOD enzyme and [C] level of TBARS. Values of 6 samples were represented as Mean ± SEM. Variance analysis (ANOVA) was performed, followed by 'Multiple Comparison Student's one-tail t-test'. Bars marked with separate superscripts (a-e) significantly (p <0.05) differ from each other

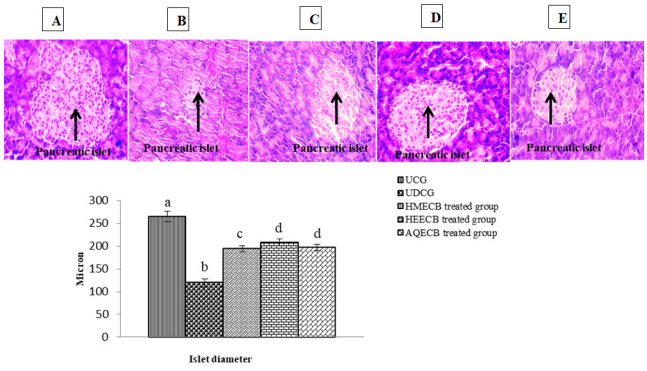

Fig. 3: Histoarchitectural restoration of pancreatic islets cell population density and their diameters after the treatment of DSECB in diabetic rat, 400X. [A] UCG [B] UDCG, [C] HMECB, [D] HEECB, [E] AQECB exposed groups. Bar diagram of islets diameter of different groups. Representation of the obtained data (n = 6) was done as Mean ± SEM. Variance analysis (ANOVA) was performed, followed by 'Multiple Comparisons one tail-t-test'. Bars with separate superscripts (a-d) significantly (p <0.05) varied from each other

been supported by previous studies.[24] Treatment with DSECB in diabetic rats enhances insulin levels, correcting glycemic status, reducing protein catabolism, prevents muscle wasting, and rectifying hyperglycemia-linked metabolic imbalances.[25]

The significant increase in FBG, as well as decreased serum insulin level of UDCG, may be due to the diabetes-associated damage of pancreatic beta cells, impairing the insulin-regulated pathway of carbohydrate metabolism, which is parallel to other studies.[26] Treatment with DSECB restored the level of FBG and serum insulin, which may be linked with phytomolecule(s), particularly flavonoid-guided inhibition in ATP-sensitive potassium channels, resulting in membrane depolarization and voltage-gated calcium ion channel activation, leads to calcium ions influx and subsequent insulin release from pancreatic β-cells. Additionally, the restoration of these levels may be due to the phytomolecule(s) of the plant extract, namely flavonoids or certain alkaloids, interacting with genes involved in pancreatic β-cells generation and function, improving insulin synthesis and secretion, aiding in the regulation of blood glucose levels.[27,28]

The key indicators of impaired glucose metabolism in diabetes include decreased HEX activity, reduced glycogen content, and increased G6Pase activity, reflecting a significant metabolic shift in diabetic rats towards diminished glucose utilization and enhanced glucose production. Decreased HEX activity in UDCG may be linked with the impairment in insulin-sensitive glucose transporters (GLUT-4), reduced glucose phosphorylation, and rising glucose-6-phosphate, as well as an elevated ATP/Mg^{2+} ratio in targeted cells.[29,30] Simultaneously, enhanced G6Pase activity in UDCG is driven by increased gluconeogenesis and glycogenolysis, mostly because of reduced insulin production and the activation of cyclic AMP and glucose-6-phosphate translocase activity, which is substantiated to other research work.[31] In addition, decreased storage of glycogen in metabolic tissue in UDCG may be due to the deficit amount of insulin secretion.[32] The treatment with DSECB leads to an elevation in HEX activity, while diminished G6Pase activity may be due to phytomolecule(s) that improve insulin sensitivity and promote cellular glucose uptake by activating the adenosine monophosphate kinase pathway, which enhanced the stimulation of GLUT- 4 translocation to the cell surface. These phytomolecule(s) may also inhibit G6Pase activity, blocking the hydrolysis of glucose-6-phosphate into glucose. This dual effect promotes enhanced glucose phosphorylation and uptake, helping to regulate gluconeogenesis and glycogenolysis pathways and thereby improving glucose metabolism.[33,34] Additionally, the DSECB treatment corrects reduced glycogen content in UDCG by stimulating glycogen synthesis. This action was most likely triggered by phytomolecule-mediated, particularly phenolic compounds activation of insulin-stimulated protein phosphatase-1, which catalyzed the dephosphorylation and subsequent activation of glycogen synthase, while inactivating glycogenolysis-regulating enzymes, such as phosphorylase a and phosphorylase kinase.[35,36] This event is further supported by previous research.[37,38]

Diabetes induces oxidative damage in liver and kidney tissues by generating excess ROS. This is accompanied by reduced kinetics of antioxidant enzymes like SOD and CAT as well as an elevated level of lipid peroxidation end product, TBARS, in UDCG.[39] Treatment with DSECB results the improvement in the said enzyme activity, which may be due to the phytocompound(s) centric upregulation

of SOD and CAT gene expression or direct scavenging of ROS or serving the cofactor for optimum activity of these enzymes or suppressing the ROS generating enzyme such as NADPH oxidase.[40] Plant phytomolecule(s) are crucial in reducing the oxidative stress associated with TBARS by exhibiting the scavenging activity ROS and enhancing the enzymatic antioxidant activity or reducing the TBA-MDA adduct formation.[41] The other plant extract can perform such kind of work in this line.[19]

The relationship between hyperlipidemia and vascular complications in diabetes is well documented earlier.[15] In this study, serum cholesterol, triglycerides, LDL-C levels were increased with decreased HDL-C levels in UDCG. The outcome of this study showed that the remarkable recovery of lipid sensors following treatment with DSECB may be due to a reduction in the availability of the free bile acid pool by phytomolecule-mediated bile acids binding in the intestine or inhibiting cholesterol biosynthesis, which is closely linked to HMG-CoA reductase. Additionally, it may be attributed to the increased uptake of LDL-C through phytomolecule-linked enhanced LDL receptor expression, or phytomolecule(s) may elevate the HDL-C levels by up-regulation of ATP-binding cassette transporter A1and lecithin cholesterol acyl transferase gene expression,[42,43] or by inhibiting pancreatic lipase activity and regulating lipoprotein lipase. All such possible factors are important for regulating the lipid profile.

Decreased beta-cell population and diameter were shown in the pancreatic histology of UDCG, which may be due to the STZ-linked destruction of pancreatic β-cell, leading to structural changes and impairing insulin secretion.[44] The DSECB treatment results in the generation of β-cells may be initiated by plant-derived compounds, which promote β-cell replication by interacting with and activating regulatory proteins such as cyclins and cyclin-dependent kinase. This facilitated the transition from the G1 to S phase of the cell cycle, triggering cell cycle progression. Furthermore, these phytocompound(s) promoted the differentiation of new β-cells from pancreatic progenitor cells, which is crucial for replenishing β-cell mass as supported by our previous study using another plant extract.[28] In this way, the phytocompound(s) in the plant extract may alleviate the damage to pancreatic tissue by restoring its structure and function and by reducing oxidative stress-induced inflammation.[45]

The activities of ACP and ALP enzymes were assessed to evaluate general toxicity. In the UDCG group, these enzyme levels increased in the serum due to hyperglycemia-related damage to cellular organelles, particularly lysosomal instability. This dysfunction can lead to the leakage of enzymes into circulation, raising their levels in the serum.[46,47] The serum level of that enzyme was decreased after DSECB treatment, which may be due to phytocompound(s) mediated stabilization of lysosomal membranes and mitigation of cellular organelle damage.[48] The increased level of key metabolite (urea, uric acid, creatinine) in the serum of UDCG reflects deleterious effects of insulin resistance, promoting significant protein breakdown, leading to glomerular dysfunction and structural damage of kidney tissue. This hinders their ability to remove waste from circulation, leading to urea and uric acid as well as creatinine level accumulation in the serum.[49] Treatment of DSECB to the diabetic rat resulted in significant amelioration of the nephrotoxicity biosensors by minimizing the protein breakdown and improving the glucose utilization, contributing to diminished levels of these metabolites, indicating that phytomolecule-linked renal function replenishment. These mechanisms can help to mitigate kidney damage caused by hyperglycemia, as supported by the previous research.[50,51]

The significant non-toxic effect of DSECB was supported by the acute toxicity study which showed no such adverse effect on behavior or any mentioned noticeable symptom that highlights the safety profile of DSECB treatment.[14]

The systematic phytochemical(s) analysis of DSECB revealed the presence of five natures of phytocompound(s), namely alkaloid, flavonoid, tannin, phenol, and terpenoid, but the intensity of phytomolecule(s) were varied in different extracts. These compounds not only contribute to the antidiabetic, antioxidative effects but also provide significant antihyperlipidemic effects.[52]

From the above analysis, it has been shown that the phytomolecule(s) of DSECB may work on multiple fronts to enhance these effects. Among three different solvent-based extracts, HEECB demonstrated superior efficacy in correcting the oxidative stress-linked diabetes-associated co-morbidity.

The phytopharmacological insight from plant extract includes the phytocomound(s) in the plant extract directly modulating the activity of carbohydrate metabolic enzymes as well as acting through the genomic interaction of enzymes involved in the carbohydrate metabolic pathway, which may help to regulate blood glucose by improving the glucose utilization at a cellular level. Additionally, phytocompound(s) ameliorate the activity of ant-oxidative markers both non-genomically and by regulating genes involved in antioxidative enzymes. This dual mechanism strengthens the body's defense against oxidative stress. Furthermore, the plant phytocompound(s) recover the lipid sensors through regulating the rate-limiting enzymes and concerning genes associated with the sensors, helping to control the hyperlipidemia and co-morbidity of diabetes. This study has some limitations from genomic analysis and specific biomolecule separation point of view. Further research is required in this line, which is ongoing in our laboratory, that could assist in developing more effective and safe antidiabetic medications and help to mitigate the associated complications.

Conclusion

The findings suggest that the HEECB is the most effective than other solvent extracts for managing diabetes and the associated complications. In the future, this extract may widen the way to develop new antidiabetic drugs through further research and analysis.

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