**Cyperus iria** aqueous-ethanol extract ameliorated hyperglycemia, oxidative stress, and regulated inflammatory cytokines in streptozotocin-induced diabetic rats

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**Abstract**

Type 2 diabetes mellitus is a complicated metabolic disorder with no definite treatment. *Cyperus iria* (Cyperaceae) possess several traditional therapeutic uses. According to the folklore tales, the whole plant of *Cyperus iria* possesses antihyperglycemic activity. The present study was undertaken to investigate whether aqueous-ethanol extract of *Cyperus iria* can ameliorate the altered activities of carbohydrate metabolism in streptozotocin (STZ)-induced diabetic rats along with appraisal of inflammatory and stress markers involved in endocrine dysfunction. Presence of biophenolics and flavonoids might be responsible for the antidiabetic potential. STZ-induced diabetic rats were treated orally with *Cyperus iria* extract (125, 250, and 500 mg/kg) for 15 days. Blood samples were collected. Metformin was used as positive control. Significantly higher quantities of phenolic (82.79 ±0.003 mg/g GAE) and flavonoid (13.61±0.002 mg/g QE) contents were present. Inhibitory concentration (IC50) exhibited an excellent potential for both antioxidant (IC50= 3.22 μg/mL) and alpha amylase (IC50=36.29 μg/mL) inhibitory assays. High-performance liquid chromatography (HPLC) confirmed the existence of myercetin, quercetin, kaempferol, and ferulic acid. *Cyperus iria* aqueous-ethanol extract exhibits good tolerance against glucose at 90 min in normal rats. Streptozotocin-induced hyperglycemia declined significantly at day 9 (265 mg/dL) along with improvement in inflammatory (TNF-α=15.6± 0.2 g/l, COX-2=357±0.396 U/l, IL-6= 572±0.99 pg/l) and oxidative stress markers (SOD= 163±0.616 and GSH-ST= 95.8±0.44 U/mL) along with biochemical parameters in a dose-dependent manner. Present study suggests that *Cyperus iria* aqueous-ethanol extract possesses hypoglycemic potential which might be attributed to the decrease in oxidative stress and inflammatory markers.

**Keywords** *Cyperus iria* • Oxidative stress • Anti-inflammatory • Streptozotocin • Antihyperglycemic

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Introduction

Diabetes mellitus (DM) is a disorder with disrupted metabolism of carbohydrates, proteins, and fats associated with hyperglycemia due to inadequate insulin production or insulin resistance. According to the World Health Organization (WHO) number of persons suffering from diabetes, the toll has risen from 108 million to 422 million in the last 2 decades (Kundury and Hathur 2020). Reduced tissue uptake results in extracellular hyperglycemia and intracellular hypoglycemia. This abnormal behavior is linked with the decreased production of insulin or due to the resistance of target organs mainly adipose tissues, skeletal muscles, and liver. The severity of symptoms is based on the type of diabetes. DM is associated with chronic damage, impairment, and failure of various organs that leads to neuropathy, retinopathy, cardiovascular problems, nephropathy, and liver diseases. The indications of hyperglycemia are blurred vision, polyuria, polyphagia, polydipsia, and weight loss. Immune-mediated diabetes may accompany improper growth and susceptibility to a variety of infections (Asmat et al. 2016). Type 2 DM is known as non-insulin dependent diabetes mellitus (NIDDM): 90–95% of individuals with diabetes suffer from NIDDM. This adult-onset diabetes is characterized by resistance to insulin and relative insulin deficiency. There are many possible causes of type 2 DM but specific etiology remains unknown. The hallmark signs of type 2 DM include frequent urination, lethargy, increased thirst, weight loss, sweating, and increased hunger (Gavin III et al. 1997). World Health Organization (WHO) defines type 2 DM as increased sugar levels on two instances: First one considers fasting plasma glucose (FPG) greater than 7.0 mmol/L. Secondly by using a glucose tolerance test (GTT), in which plasma glucose level of 11.1 mmol/L was considered as a threshold after 2 h of oral glucose administration (Ginter and Simko 2013).

Different glucose lowering agents are available for the treatment of DM. The selection of the medicinal agent is based upon the prevalence of the disease, age, sex, body mass index, and other disease factors (Peters and Davidson 1996). These agents include the drugs that increase the production of insulin from pancreas (glimepiride, repaglinide, glipizide, chlorpropamide, glyburide, nateglinide), drugs that reduce absorption of glucose from intestines (acarbose, miglitol), drugs that cause improvement in utilization of insulin (rosiglitazone, pioglitazone), drugs that lessen the production of glucose by the liver and help in improvement of insulin resistance (metformin), drugs that raise insulin production from pancreas or decrease production of glucose by liver (dulaglutide, linagliptin, exenatide, saxagliptin, alogliptin, liraglutide, semaglutide, lixisenatide, sitagliptin), drugs that inhibit glucose reabsorption by kidney and enhance excretion of glucose in urine called sodium glucose transport (SGLT)2 inhibitors (dapagliflozin, canagliflozin, empagliflozin). Another medicinal agent is Pramlinitide which is a synthetic hormone that helps in lowering blood glucose (Control and Group CTR 1995; Sabry et al. 2019).

A wide range of plants possess medicinal properties (Barkaoui et al. 2017). Almost 50% of the current medicines being used are herbal based (Kamboj 2000). Increase in the incidence of diabetes encompasses new patients with altogether different physiology and issues related to drug metabolisms that surfaces new drug-induced adverse reactions that were hitherto unknown to the scientific community and pose a challenge to find safer alternatives. World Health Organization (WHO) has approved the usage of conventional plants for the management of DM because they are nontoxic and shows lesser or no adverse reactions. This is because they are considered to be worthy candidates to be used as an oral treatment (Manukumar et al. 2017).

Oxidative stress and inflammation are acknowledged to play a pivotal role in the pathogenesis of type 2 DM and in its complications (Oguntibeju 2019; Halim and Halim 2019; Charlton et al. 2021). Reactive oxygen species are involved in the incidence of type 2 DM, owing to unstable nature of free radicals (Abdulwahab et al. 2021). Noenzymatic glycation of proteins, glucose oxidation, and augmented lipid peroxidation generates free radicals that further damage enzymes, cellular machinery and develop insulin resistance due to oxidative stress (Lugrin et al. 2014). Oxidative stress can also lead to systemic inflammation that contributes to the pathophysiology of many macrovascular and microvascular complications of type 2 DM. Pro-inflammatory cytokines like interleukin-6 (IL-6) levels were significantly higher among type 2 DM patients than among disease-free controls. It suggests a potential role for IL-6 in the development and progression of type 2 DM.

*Cyperus iria,* locally known as rice flatsedge, belongs to family Cyperaceae. *Cyperus iria* is 1–2 mm in width and can reach up to 60 cm in height. It possesses trigonal, thin, and smooth stems (Semwal et al. 2016). Different synonyms for *Cyperus iria* are *Chlorocyperus iria,* *Cyperus microiria,* *Cyperus microlepis,* *Cyperus panicoides,* and *Cyperus santonic.* According to the folklore tales, the whole plant of *Cyperus iria* possesses antihyperglycemic activity (Banik et al. 2010). Previously, the plant has been reported to possess potent in vitro antioxidant activities using different assays (Ho et al. 2012). Based upon the folktales and reported in vitro antioxidant activities, it was hypothesized that *Cyperus iria* ameliorates hyperglycemia owing to the presence of phenolic and flavonoids. Present study was designed to evaluate the effect of aqueous-ethanol *Cyperus iria* extract on the carbohydrate metabolism linked with oxidative stress and inflammatory biomarkers in streptozotocin (STZ)-induced diabetic rats. To the best of our knowledge, no study was conducted in current aspect of its antihyperglycemic potential till date.
Materials and methods

Plant gathering and authentication

The whole plant of *Cyperus iria* was gathered from different rice fields and wetlands of Lahore, Pakistan, during month of May and June 2018. Plant sample was authenticated by Botany Department of Government College University, Lahore. A voucher with specimen number GC.Herb.Bot.3526 was deposited in the department’s herbarium unit for future reference. The gathered plant was thoroughly washed and air-dried in shade. After 15 days, the dried plant was grounded to powder with the help of an electronic herbal grinder (LX-10A, 1800 W, Shanghai Jiang Xin Technology Co. Ltd, China).

Preparation of plant extract

Extract was prepared through cold maceration using ethanol and distilled water (1:3 w/v), named as aqueous-ethanol extract of *Cyperus iria*. The filtered extract was dried in rotary evaporator and oven. The dried crude extract was reserved in glass vials for further investigation (Malik et al. 2020).

Phytochemical analysis

The aqueous-ethanol extract of *Cyperus iria* was tested for phytochemicals according to the protocols described previously by Zaib et al. (2020).

Total phenolic contents

Total phenolic contents were estimated by Folin Ciocalteu’s method (Zaib et al. 2020). Different dilutions (20, 40, 60, 80, 100, and 120mg/mL) of gallic acid and aqueous-ethanol extract of *Cyperus iria* were prepared. The experiment was performed in triplicate. Absorbance was measured at 760nm using UV spectrophotometer (UV-1700, Shimadzu). The phenolic contents were measured as gallic acid equivalent (GAE) and expressed as (GAE mg/g of *Cyperus iria* aqueous-ethanol extract).

Total flavonoid contents

Total flavonoid content was measured with the aluminum chloride colorimetric assay (Ahmed et al. 2017). Different dilutions (20, 40, 60, 80, 100, and 120mg/mL) of standard quercetin and aqueous-ethanol extract of *Cyperus iria* were prepared from the stock solution. The absorbance was measured at 415 nm using UV-visible spectrophotometer (UV-1700, Shimadzu). Flavonoid contents were measured as quercetin equivalent (QE) and expressed as QE mg/g of *Cyperus iria* aqueous-ethanol extract (Zulfqar et al. 2020).

High-performance liquid chromatography (HPLC) analysis

The phytochemical quantification of aqueous-ethanol extract of *Cyperus iria* was conducted using HPLC system LC-10 Shimadzu (Kyoto, Japan). HPLC system was equipped with a photodiode array (PDA) detector SPD-10AV and a UV-visible detector (280 nm wavelength) along with pump (LC-10AT). A CLC-ODS(C-18) shim pack column (25cm×4.6mm, 5μm) was used. Two mobile phases A (water; acetic acid (94:6, pH 2.27) and B (100% acetonitrile) with a flow rate of 1mL/min were used as gradients. The sample results were evaluated by comparison with the standard peaks used as reference (Zafar et al. 2020).

The concentration of detected flavonolic and phenolic compounds were calculated from the calibration curve by formula:

\[
\text{concentration of sample} = \frac{\text{Area of sample}}{\text{Area of standard}} \times \text{concentration of standard}
\]

Investigation of in vitro antioxidant activity by DPPH assay

The antioxidant capacity of aqueous-ethanol *Cyperus iria* extract was carried out by using DPPH assay (Razzaque et al. 2020) to determine its antioxidant potential. Different dilutions of 20, 40, 60, 80, 100, and 120mg/mL were prepared from the stock solution of plant extract and ascorbic acid. The absorbance was measured by UV spectrophotometer (UV-1700, Shimadzu) at wavelength of 517nm. The inhibitory concentration was calculated from prepared inhibition curve. Radical scavenging activity was measured by

\[
\%\text{age inhibition} = \frac{\text{Positive control} - \text{standard or extract}}{\text{Positive control}} \times 100
\]

Investigation of in vitro antioxidant activity by phosphomolybdenum reduction assay

The total antioxidant capacity (TAC) of aqueous-ethanol extract of *Cyperus iria* was analyzed using phosphomolybdenum complex forming assay. Different dilutions of concentrations 20, 40, 60, 80, 100, and 120mg/mL were prepared from the stock solutions of plant extract and standard ascorbic acid. The absorbance of the samples was measured at 695nm using a UV spectrophotometer (UV-1700, Shimadzu). The antioxidant capacity was measured and expressed in μg of ascorbic acid equivalents (AAE) per mL.
Estimation of in vitro inhibitory α-amylase assay

In vitro antidiabetic activity was estimated using α-amylase inhibition assay. The plant extract and standard acarbose solutions were prepared in different concentrations of 10, 20, 40, 60, 80, 100, and 120 mg/mL. Absorbance was measured at 540 nm (Razzaque et al. 2020). The inhibitory concentration was calculated from prepared inhibition curve. The percentage inhibition of α-amylase activity was measured by

\[
\text{Inhibitory activity of } \alpha \text{ amylase} = \frac{A_c - A_e \text{ or } A_s}{A_c} \times 100
\]

where \(A_c\) is the control’s absorbance, \(A_s\) standard’s absorbance, and \(A_e\) extract’s absorbance.

Experimental animals

Albino–Wistar rats of either gender (200–250g) were kept in cages under standard conditions (20–25°C) and fed with standard pellet food and water ad libitum. An ethical approval of the experimental protocol was obtained from the Institutional Research Ethics Committee (IREC-2018-63) of the University of Lahore and the experiments were conducted according to these institutional guidelines.

Acute toxicity study

Acute oral toxicity was performed to determine the dose levels according to the Organization of Economic Cooperation and Development (OECD) 423 guidelines. Wistar rats were divided into 4 groups. Rats were administered with single dose of 200, 2000, and 5000 mg/kg aqueous-ethanol extract of Cyperus iria each along with normal control group. The dose was administered in 12-h fasted rats through oral gavage. After administration, the rats were carefully observed for behavioral patterns at 0.5, 4, 16, 24, and 48 h. After 48 h, the animals were dissected for the histopathological examination of liver and pancreas.

Oral glucose tolerance test (OGTT)

The test was performed to determine the tolerance of the animal against glucose when administered in fasting condition. Wistar rats fasted for 24h were selected. The blood glucose level was measured and hyperglycemia was induced with glucose solution, followed by the administration of metformin (500 mg/kg) and extract at dose levels of 125, 250, and 500 mg/kg BW. Glucose level was monitored at 0 30, 60, 90, 120, 180, and 210 min by withdrawing blood sample from the tip of tail vein using Accu-Check glucometer (Roche). Antihyperglycemic inhibition was calculated following the protocol of Amuri et al. (2017).

Experimental design

Negative control group (n=5) received normal saline (1 mL/kg) orally. Diabetic control group (n=5) diabetes induced by streptozotocin (STZ) (55 mg/kg), nicotinamide (110 mg/kg) intraperitoneal (Zafar et al. 2020). Positive control group (n=5) administered metformin orally in dose of 100 mg/kg after inducing diabetes (Mazumder et al. 2021). CI 125 mg/kg: Treatment group (n=5) administered 125 mg/kg aqueous-ethanol extract of Cyperus iria after inducing diabetes. CI 250 mg/kg: Treatment group (n=5) that were administered 250 mg/kg aqueous-ethanol extract of Cyperus iria after inducing diabetes. CI 500 mg/kg: Treatment group (n=5) that were administered 500 mg/kg aqueous-ethanol extract of Cyperus iria after inducing diabetes.

Induction of diabetes

Wistar rats were starved overnight. Freshly prepared nicotinamide (110 mg/kg BW) dissolved in normal saline was administered intraperitoneal (IP) and after a 15-min interval STZ (55 mg/kg BW) dissolved in 0.1 molar (M) citrate buffer (pH 4.5) was injected in a single dose intraperitoneally. The rats were given 5% dextrose solution overnight after the STZ administration to avoid hypoglycemic shock. The blood glucose levels were assessed by drawing blood from the tail vein by an Accu-Check glucometer (Roche). After 72 h of STZ administration, the rats having glucose level exceeding 250 mg/dL were selected for the study and considered diabetic (Zafar et al. 2020).

Antihyperglycemic activity

The activity was performed to determine the effect of treatments against STZ-induced diabetes. After the induction of diabetes, the rats were monitored for 15 days. The rats were given their accurate, freshly prepared doses by an oral gavage tube, once a day. The fasting blood glucose levels were checked at 0, 3, 6, 9, 12, and 15 days using an Accu-Check glucometer (Roche, Switzerland).

Estimation of inflammatory cytokines

Pro-inflammatory cytokines were measured in the serum samples of all treated groups to establish the link between type 2 DM and inflammation. Rat Eliza kits K1052, K4145, and E4801 were used to evaluate tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6), and cyclooxygenase-2 (COX-2) respectively according to manufacturer instructions (BioVision Inc, California). The serum samples were allowed to clot for 30 min in serum separator tubes followed by centrifugation at 3000×g for 10 min (Malik et al. 2020).
Estimation of antioxidant enzymes

The activity of defense antioxidants superoxide dismutase (SOD) and glutathione S-transferase (GSH-ST) was measured in plasma samples of animals to determine the oxidative stress using commercial kits obtained from Nanjing Jiancheng Bioengineering Institute China as per manufacturer’s instructions. Blood sample (3 mL) was collected in heparinized tubes from each rat. It was centrifuged for 10 min at 1000 rpm at 4 °C. Plasma as supernatant (yellow layer) was cautiously drawn and positioned on ice pack (Sharif et al. 2016b).

Biochemical parameters

After the 15-days study, the rats were fasted overnight and given diethyl ether anesthesia. The rats were sacrificed by cervical decapitation. Blood samples were collected by cardiac puncture and stored in sterile tubes. Blood was collected in EDTA tubes for analysis of parameters. The blood samples were left for 2 h at room temperature, centrifuged for 15 min at 1500×g at 4°C. The supernatant was instantly separated from the pellet and serum samples were prepared in order to estimate the triglyceride level (TG), total cholesterol level (TC), high-density lipoproteins (HDL), low-density lipoproteins (LDL), very low-density lipoproteins (v-LDL), alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), urea, and creatinine. The samples were submitted to UOL diagnostic lab and research center for biochemical analysis (Khan et al. 2020).

Histopathology

The liver, kidney, and pancreas of treated and control groups were dissected out and rinsed with ice-cold saline solution. These organs were preserved in 10% neutral formalin solution. Paraffin blocks were prepared after being washed, dehydrated with alcohol, and cleared by xylene. A rotary microtome was used for cutting sections with a thickness of 4–5 mm and stained with hematoxylin and eosin. The randomly selected fields of view were observed for histopathological changes under light microscope for any signs of toxicity after treatment (Optika Vision Lite 2.1) (Sharif et al. 2016a). The histological sections were analyzed by a pathologist who was blinded to the treatment groups. The damage to the tissues was scored according to a scoring system mentioned in Table 1. The scoring system has a total of five grades according to the severity of the damage ranging from 0 to 4. The severity of the kidney injury includes inflammation, glomerular thickening, and tubular degeneration. Hepatic alterations include inflammation, necrosis, fibrosis, and steatosis whereas pancreatic injury includes inflammation, degeneration of acinar cells, and degeneration of β-cells. Injuries were graded as follows for each criterion: < 1%, 0; 1 to 25%, 1; 26 to 50%, 2; 51 to 75 %, 3 and > 75%, 4.

The results are expressed as mean ± SD. The IC_{50} was calculated by nonlinear regression model using Graph Pad Prism 6.0. Significant difference was assessed between diabetic control and experimental groups. One-way analysis of variance (ANOVA) was performed using Graph Pad Prism 5.0 software followed by Tukey’s test and probability level p<0.05 was considered statistically significant. The oral glucose tolerance test was evaluated by measuring area under the curve (AUC).

Results

Phytochemical analysis

Phytochemical screening was performed using conventional qualitative tests. The analysis revealed the presence of alkaloids, glycosides, flavonoids, phenols, carbohydrates, fixed oils, proteins, and terpenoids in the aqueous-ethanol extract of Cyperus iria.
The ultraviolet (UV) spectrophotometry was used to quantify the amount of polyphenols present in the aqueous-ethanol extract of *Cyperus iria*. A calibration curve was established using different concentrations of gallic acid from a stock solution of 100 μg/mL to estimate the phenolic contents of the aqueous-ethanol extract of *Cyperus iria*. The concentration of the phenolic compounds was then calculated from the calibration curve and expressed in milligrams of gallic acid equivalent mg/g GAE. The absorbance of the sample of the aqueous-ethanol extract was related to the concentration of the polyphenols in the aqueous-ethanol extract of *Cyperus iria*, using the linear equation $y=0.0119x+0.1577$, $R^2 = 0.9849$ defined by the gallic acid calibration curve. The total phenolic contents in aqueous-ethanol extract of *Cyperus iria* were found to be 82.79 mg/g GAE.

### Total flavonoid contents

The flavonoid calibration curve was established using different concentrations of quercetin, a well-known flavonoid of the flavonol family. The flavonoid content of the extract was determined using the AlCl₃ method, and reference to the calibration curve described by the linear equation $y=0.0261x+0.0083$, $R^2 = 0.9775$. The total flavonoid contents in aqueous-ethanol extract of *Cyperus iria* were found out to be 13.61 mg/g QE.

### High-performance liquid chromatography (HPLC) analysis

The aqueous-ethanol extract of *Cyperus iria* was analyzed by HPLC and several constituents in the HPLC chromatogram have been identified. The UV-detectable components of aqueous-ethanol extract of *Cyperus iria* exist mainly in 5–20 min (supplementary information S1). Four components were identified, which are myercetin (0.0037ppm), quercetin (1.757ppm), kaempferol (0.0318ppm), ferulic acid (6.00 ppm) in aqueous-ethanol extract of *Cyperus iria*. These results provide a material base for the antidiabetic effect of aqueous-ethanol extract of *Cyperus iria* in diabetes treatment.

### Investigation of in vitro antioxidant activity by DPPH assay

The antioxidant capacities of aqueous-ethanol extract of *Cyperus iria* at all tested concentrations (10–100 μg/mL) were evaluated by the most commonly used antioxidant assay: DPPH radical method. The results clearly indicated that the aqueous-ethanol extract of *Cyperus iria* inhibited free radical’s generation based on concentrations used (Table 3). The scavenging activity of DPPH radical increased proportionally with extract concentration from 10–100 μg/mL in aqueous-ethanol extract of *Cyperus iria*. Inhibitory concentration of aqueous-ethanol extract of *Cyperus iria* was found out to be 39.88 μg/mL. Ascorbic acid was used as standard (IC₅₀=3.22 μg/mL).

### Table 1

| Sr no. | Organ | Item                  | Score | Grading |
|-------|-------|-----------------------|-------|---------|
| 1     | Liver | Inflammation          | < 1%  | 0       |
|       |       | 1 to 25%              | 1     |         |
|       |       | 26 to 50%             | 2     |         |
|       |       | 51 to 75%             | 3     |         |
|       |       | > 75%                 | 4     |         |
|       | Necrosis |                   | < 1%  | 0       |
|       |       | 1 to 25%              | 1     |         |
|       |       | 26 to 50%             | 2     |         |
|       |       | 51 to 75%             | 3     |         |
|       |       | > 75%                 | 4     |         |
|       | Fibrosis |                  | < 1%  | 0       |
|       |       | 1 to 25%              | 1     |         |
|       |       | 26 to 50%             | 2     |         |
|       |       | 51 to 75%             | 3     |         |
|       |       | > 75%                 | 4     |         |
|       | Steatosis |                | < 1%  | 0       |
|       |       | 1 to 25%              | 1     |         |
|       |       | 26 to 50%             | 2     |         |
|       |       | 51 to 75%             | 3     |         |
|       |       | > 75%                 | 4     |         |
|       | Kidney | Inflammation          | < 1%  | 0       |
|       |       | 1 to 25%              | 1     |         |
|       |       | 26 to 50%             | 2     |         |
|       |       | 51 to 75%             | 3     |         |
|       |       | > 75%                 | 4     |         |
|       | Glomerular thickening |            | < 1%  | 0       |
|       |       | 1 to 25%              | 1     |         |
|       |       | 26 to 50%             | 2     |         |
|       |       | 51 to 75%             | 3     |         |
|       |       | > 75%                 | 4     |         |
|       | Tubular degeneration |             | < 1%  | 0       |
|       |       | 1 to 25%              | 1     |         |
|       |       | 26 to 50%             | 2     |         |
|       |       | 51 to 75%             | 3     |         |
|       |       | > 75%                 | 4     |         |
|       | Pancreas | Inflammation         | < 1%  | 0       |
|       |       | 1 to 25%              | 1     |         |
|       |       | 26 to 50%             | 2     |         |
|       |       | 51 to 75%             | 3     |         |
|       |       | > 75%                 | 4     |         |
|       | Degeneration of acinar cells | | < 1%  | 0       |
|       |       | 1 to 25%              | 1     |         |
|       |       | 26 to 50%             | 2     |         |
|       |       | 51 to 75%             | 3     |         |
|       |       | > 75%                 | 4     |         |
|       | Degeneration of Beta cells | | < 1%  | 0       |
|       |       | 1 to 25%              | 1     |         |
|       |       | 26 to 50%             | 2     |         |
|       |       | 51 to 75%             | 3     |         |
|       |       | > 75%                 | 4     |         |
Investigation of in vitro antioxidant activity by phosphomolybdenum reduction assay

The reactivity of aqueous-ethanol extract of *Cyperus iria* is evaluated for measuring total antioxidant capacity by the phosphomolybdenum method. The assay method is based on the reduction of Mo (VI) to Mo (V) by the biological sample followed by formation of a green phosphate Mo (V) complex at acidic pH. TAC of aqueous-ethanol extract of *Cyperus iria* was found to be 20.04 mg/g ascorbic acid equivalent (AAE). Regression equation was used to determine TAC: \( y = 0.0072x - 0.0319 \), \( R^2 = 0.977 \).

Estimation of in vitro inhibitory alpha-amylase assay

Acarbose produced a concentration-dependent inhibition of \( \alpha \)-amylase enzyme. All concentrations of aqueous-ethanol extract of *Cyperus iria* were tested on \( \alpha \)-amylase enzyme. Inhibitory concentration of aqueous-ethanol extract of *Cyperus iria* for alpha amylase was found out to be 36.29 \( \mu \)g/mL. Acarbose was used as standard (IC50=5.710 \( \mu \)g/mL). Results are depicted in Table 4.

Acute toxicity study

No mortality in rats was observed up to 5000 mg/kg dose of *Cyperus iria* aqueous-ethanol extract in acute toxicity study. However, liver and kidney section revealed moderate degeneration of hepatocytic cells and \( \beta \)-cells, respectively. At the dose of 2000mg/kg, histopathological scores were non-significant when compared to normal control group but administration of 5000mg/kg dose of aqueous-ethanol extract of *Cyperus iria* exhibited significant difference in inflammation \( (p<0.001) \) whereas necrosis, fibrosis, and steatosis found to be less significant \( (p<0.05) \) on comparison with normal control group Figure 1A. Pancreatic scoring exhibited a significant difference \( (p<0.05) \) only in inflammation at the dose of 2000mg/kg but at 5000mg/kg dose of aqueous-ethanol extract of *Cyperus iria* inflammation differed significantly \( (p<0.001) \) whereas degeneration of acinar and \( \beta \)-cells was less significant \( (p<0.05) \) with respect to normal control group Figure 1B. Histopathological changes are depicted in Figure 2A and B.

Oral glucose tolerance test (OGTT)

Oral glucose tolerance test was performed on normoglycemic rats. Area under curve (AUC) was calculated by plotting concentration of glucose against time interval. Based on the mean of the highest glycemic values (Cmax) of each experimental group, the hyperglycemic inhibition rate was determined by comparison with the mean peak value of the glucose group. All tested concentrations induced a sensible reduction in glucose AUC and Cmax, an effect practically comparable with the one observed for glibenclamide. The aqueous-ethanol extract of *Cyperus iria* at the concentration 500mg/kg exhibited maximum antihyperglycemic inhibition of 15.45% achieving

### Table 2 Phyto-constituents identified in aqueous-ethanol extract of *Cyperus iria*

| Phyto-constituents | Concentration (ppm) | Retention time (min) | Area (mAU*s) | Area (%) |
|-------------------|--------------------|---------------------|--------------|----------|
| Flavonoid compounds |                    |                     |              |          |
| Myrcetin          | 0.0037             | 4.16                | 2.23673      | 3.3315   |
| Quercetin         | 1.754              | 5.793               | 55.88739     | 83.2402  |
| Kaempferol        | 0.0318             | 8.413               | 9.01580      | 13.4284  |
| Phenolic compound |                    |                     |              |          |
| Fennic acid       | 6.00               | 4.534               | 103.82788    | 100.00   |

### Table 3 %age inhibition of DPPH analysis at different concentrations of aqueous-ethanol extract of *Cyperus iria* and IC50 values

| Concentration (\( \mu \)g/mL) | %age inhibition of ascorbic acid | IC50 value (\( \mu \)g/mL) | %age inhibition of aqueous-ethanol extract of *Cyperus iria* | IC50 value (\( \mu \)g/mL) |
|-------------------------------|---------------------------------|--------------------------|----------------------------------------------------------|--------------------------|
| 10                            | 74.00±0.176                    | 3.22                     | 75.40±0.055                                               | 39.88                    |
| 20                            | 78.04±0.001                    | 7.840±0.006              | 79.50±0.016                                               | 80.18±0.003              |
| 40                            | 79.03±0.001                    | 80.18±0.003              | 81.20±0.004                                               | 83.00±0.002              |
| 60                            | 79.60±0.006                    | 81.20±0.004              |                                                          |                          |
| 80                            | 80.00±0.010                    | 83.00±0.002              |                                                          |                          |
| 100                           | 82.09±0.005                    |                          |                                                          |                          |
a Cmax of 244 mg/dL within 60 min, which is close to the positive control group. At the end of the study, the glucose concentration was 98.6 mg/dL which is comparable to positive control 96.8 mg/dL (Figure 3A).

**Antihyperglycemic activity**

The blood glucose level was measured in normal and experimental groups at 0, 3rd, 6th, 9th, 12th, and 15th day of treatment. STZ administration showed a significant increase ($p < 0.001$) in the blood glucose level when compared to normal control group. Dose-dependent decrease in hyperglycemia was observed when rats were treated with different concentrations of aqueous-ethanol extract of *Cyperus iria*. At day 15, glucose concentration was decreased to 199 mg/dL at the dose of 500 mg/kg as compared to 183 mg/dL for positive control. The results were analyzed by means of one-way analysis of variance (ANOVA) which displayed statistically significant difference ($p <0.001$) in contrast with the diabetic control group (Figure 4).

**Estimation of inflammatory cytokines**

Concentrations of pro-inflammatory mediators were raised in STZ-induced diabetic rats when compared to normal control.
group. The induction of diabetes in rats increased serum TNF-α, COX-2, and IL-6 concentrations reaching 22.5 ±0.15 g/l, 458±4.7 U/l, and 1195±16.4 pg/l respectively compared to the normal group. Their release was markedly inhibited as a result of aqueous-ethanol extract of *Cyperus iria* administration. Upon treatment with aqueous-ethanol extract of *Cyperus iria*, levels of TNF-α, IL-6, and COX-2 were reduced to 15.6±0.2 g/l, 357±0.39 U/l, and 572±0.99 pg/l respectively exhibiting an improvement in the levels of inflammatory cytokines. A dose-dependent effect was observed (Figure 5A, B, C).

**Estimation of antioxidant enzymes**

The present study revealed significant reductions (*p < 0.001*) in SOD and GSH-ST activities in STZ-induced diabetic rats when compared to normal rats. The administration of aqueous-ethanol extract of *Cyperus iria* increased the activities to 163±0.616 U/ml and 95.8±0.44 U/ml in plasma, respectively, when compared to the activity in the positive control rats (*p <0.001*). Aqueous-ethanol extract of *Cyperus iria* restored the SOD and GSH-ST activities in treated rats in a dose-dependent fashion (Figure 5D, E)
Biochemical parameters

Biochemical parameters are severely affected by oxidative stress, which result in damages to deoxyribonucleic acid (DNA) and cellular proteins. Adverse effects of dyslipidemia due to insulin resistance including lipoprotein oxidation, production of triglyceride-rich vLDL particles and hypertriglyceridermia and changing in lipoprotein metabolism are the other reasons of diabetes complications. Hepatic parameters ALT, AST, and ALP were raised to 47.8±1.11, 144±0.81, and 91.2±0.86 (U/l), respectively. Treatment with aqueous-ethanol extract of *Cyperus iria* restored the enzyme levels (p<0.001) to normal. A significant (p < 0.001) elevation in the serum levels of urea (49 ±0.54mg/dL) and creatinine (0.968±0.01mg/dL) was also observed. Treatment of the diabetic animals with aqueous-ethanol extract of *Cyperus iria* significantly (p < 0.001) decreased serum urea and creatinine concentrations. Diabetes caused a significant (p < 0.001) elevation in the serum levels of TG (132±1.52 mg/dL), TC (205±2.42 mg/dL), and LDL (171±2.68 mg/dL) and the decrease in serum level of HDL (16±0.447 mg/dL). Treatment of the diabetic animals with aqueous-ethanol extract of *Cyperus iria* significantly (p < 0.001) decreased serum TG, TC, and LDL concentration when compared to diabetic control and an increase in HDL levels was observed. Metformin administration elicited significant changes in all the lipid parameters analyzed when compared with diabetic controls. Thus, present study indicated that aqueous-ethanol extract of *Cyperus iria* reduced the levels of alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT) depicted in Figure 3 B, triglycerides (TG), low-density lipoproteins (LDL), total cholesterol (TC) (Table 5) urea and creatinine (Table 6) insulin, and C-reactive proteins (CRP) depicted in Figure 5 (F, G) in STZ-induced diabetic rats. An increase in high-density lipoproteins (HDL) and insulin levels was observed.

Histopathology

Histological alterations presented by diabetic control group were reversed significantly upon treatment with *Cyperus iria* aqueous-ethanol extract. Hepatic inflammation, necrosis, fibrosis, and steatosis were lowered in normal control group. In disease control group, we found increased hepatic inflammation, necrosis, fibrosis, and steatosis. Inflammation and necrosis were significantly higher in disease control group relative to all other groups. However, after 15-days treatment with *Cyperus iria* aqueous-ethanol extract, there was no significant reduction in hepatic inflammation, necrosis, fibrosis, and steatosis when compared to disease control group Figure 6A. Similarly, no histopathological changes were observed in normal control group regarding renal and pancreatic tissues. Disturbed architecture of renal tissue was observed after STZ challenge, which was interpreted as inflammation, glomerular thickening, and tubular degeneration and in pancreatic tissue as inflammation, degeneration of acinar, and pancreatic cells Figure 6(B,C). The altered changes were recovered upon treatment with *Cyperus iria* aqueous-ethanol extract at 15th day when compared with normal control group in form of restoration of normal architectures of the tissues with significant reductions in the scores of inflammation, glomerular thickening, and tubular degeneration along with improvements in the score of pancreatic inflammation with restoration of degenerated acinar and pancreatic cells.

Results are depicted in Figure 7(1, 2, 3). The outcomes thus exhibit that *Cyperus iria* aqueous-ethanol extract significantly reduced histopathologic changes of STZ injection in rats.

Discussion

Diabetes mellitus is one of the international health crises of the twenty-first century. Four hundred and twenty-five million individuals have been suffering from diabetes (aged 20–79 years) in addition to 352 million people with impaired glucose tolerance, which places these individuals at serious risk (Rajasekhar et al. 2019). Even though plenty of medications are available for the treatment of diabetes, they are having limitations due to their adverse side effects and high costs. Hence, it was found to be very difficult to manage and cure this disease effectively by using the available oral hypoglycemic agents (drugs) or insulin. Therefore, the focus of scientists

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**Table 4** %age inhibition of α-amylase analysis at different concentrations of aqueous-ethanol extract of *Cyperus iria* and IC50 values

| Concentration (μg/mL) | %age inhibition of acarbose | IC50 value (μg/mL) | %age inhibition of aqueous-ethanol extract of *Cyperus iria* | IC50 value (μg/mL) |
|-----------------------|-----------------------------|--------------------|----------------------------------------------------------|--------------------|
| 10                    | 71.875±0.024                | 5.710              | 3.125±0.001                                              | 36.29              |
| 20                    | 81.400±0.003                |                    | 8.590±0.008                                              |                    |
| 40                    | 92.960±0.003                |                    | 15.625±0.001                                             |                    |
| 60                    | 93.750±0.001                |                    | 20.310±0.007                                             |                    |
| 80                    | 94.530±0.003                |                    | 22.650±0.008                                             |                    |
| 100                   | 96.090±0.005                |                    | 24.210±0.006                                             |                    |
shifted towards the development of the natural herbal medicine with high therapeutic potential and less or no toxic effect. In this connection, antihyperglycemic, antioxidant, and anti-inflammatory properties of *Cyperus iria* were established in the present study. High levels of flavonoids and polyphenols were detected in the *Cyperus iria* aqueous-ethanol extract in the present study. Contrary to previous findings, present study interestingly exhibited more TPC and lesser TFC as compared to *Cyperus rotundus*. Likewise, the DPPH assay results also contradicted with previous findings and IC50 in the current study was much higher (39.88 μg/mL) than the previously reported (2.6 μg/mL) in *Cyperus rotundus* rhizome (Kamala et al. 2018). Risk of free radical mediated diseases like diabetes mellitus can be improved by consumption of antioxidants. Phenolic substances exhibited strong antioxidant properties probably by inactivation of lipid free radicals (Ismail et al. 2004). Detection of phenolic acids and flavonoids has also been reported previously in *Cyperus* genus (Augustus et al. 2015).

HPLC analysis confirmed the presence of myrcetin, quercetin, kaempferol, and ferulic acid in *Cyperus iria* aqueous-ethanol extract. These compounds are widely reported as potent antioxidants. Present study exhibited a high reducing capacity in phosphomolybdenum reduction assay. It is attributed to the scavenging of free radicals. This antioxidant capacity can be linked to the presence of unique functional groups of myrcetin that includes pyrone and two phenyl rings (Barzegar 2016).

In the present study, we revealed that the aqueous-ethanol extract of *Cyperus iria* possesses the potential to inhibit alpha amylase enzyme. Different concentrations of aqueous-ethanol extract of *Cyperus iria* significantly inhibited the activity of α-amylase. Our extract exhibited a dose-dependent inhibitory activity on digestive enzyme. So delaying the digestion of carbohydrates by inhibiting carbohydrate enzyme α-amylase in intestine resulted in decrease in glucose absorption rate consequently blunting postprandial rise in glucose. Presence of phenolics and flavonoids in the aqueous-ethanol extract of

| Parameter         | Mean test values (mg/dL) |
|-------------------|--------------------------|
|                   | Negative control group   | Positive control group | Diabetic control group | CI 125mg/kg | CI 250mg/kg | CI 500mg/kg |
| Total lipids      | 347±1.17                 | 374±1.46                | 448±1.02                | 422±0.860** | 400±2.13*** | 388±1.98*** |
| Cholesterol       | 71.4±1.50                | 86.2±1.50               | 205±2.42                | 133±1.10*** | 123±1.02*** | 98.8±0.73*** |
| Triglycerides     | 76.2±1.28                | 86.2±0.583              | 132±1.52                | 121±0.374*  | 115±0.812** | 101±1.38*** |
| HDL               | 28.8±0.374               | 22.8±0.735              | 16±0.447                | 17.6±0.678* | 18.8±1.30*  | 21±0.548*** |
| LDL               | 34±1.18                  | 54±1.30                 | 171±2.68                | 128±2.35*** | 93.4±2.16*** | 67±0.89***  |
| VLDL              | 17.6±0.5                 | 29.6±0.6                | 32.8±0.58               | 36.6±0.51   | 34.4±0.678  | 32±0.316    |

Values are expressed as Mean ± SEM (n=5)

*Shows p<0.05, **p<0.01, ***p<0.001 in comparison to diabetic control group, DCG

Figure 6 Effect of *Cyperus iria* aqueous-ethanol extract on STZ induced model of type 2 diabetes induced changes in A hepatic, B renal, C pancreatic histopathological scoring

### Table 5 Effect of *Cyperus iria* aqueous-ethanol extract on lipid profile of streptozotocin-induced diabetic rats

| Parameter         | CI 125mg/kg | CI 250mg/kg | CI 500mg/kg |
|-------------------|-------------|-------------|-------------|
| Total lipids      |             |             |             |
| Cholesterol       |             |             |             |
| Triglycerides     |             |             |             |
| HDL               |             |             |             |
| LDL               |             |             |             |
| VLDL              |             |             |             |
Cyperus iria is attributed to the inhibition of alpha amylase. Phenolics possess the ability to form lactones which can react with nucleophilic groups of alpha amylase molecules whereas flavonoids lead to enhanced insulin release due to inhibition of alpha amylase (Zafar et al. 2020). Present study is supported by earlier findings where hexahydroxyflavane and cassigarol E, isolated from Cyperus rodentus and Cyperus esculentus, were found to exhibit potential against alpha amylase inhibition (Kamala et al. 2018; Tran et al. 2014; Udogadi and Onyenibe 2019).

Eosinophilic hyaline casts were present in the tubular area. There was evidence of focal degeneration of proximal tubules. No renal tissue necrosis, tubular degeneration, fibrosis, atypia, or malignancy was observed. The Islets of Langerhans contained normal looking β-cells concentration. The acinar cells also appeared normal; B diabetic control revealed congestion of vasculature and evident peripancreatitis. β-cells were surrounded by aggregates of inflammatory cells. No evidence of atypia or malignancy was observed; C After treatment with CI 500mg/kg aqueous-ethanol extract of Cyperus iria, pancreatic tissue revealed normal looking exocrine elements. Islet of Langerhans was present in the endocrine element having moderate number of β-cells and acinar cells concentration. Degeneration of β-cells was minimal. Any evidence of inflammation, calcification, granuloma, and malignancy was not observed by earlier findings where hexahydroxyflavane and cassigarol E, isolated from Cyperus rodentus and Cyperus esculentus, were found to exhibit potential against alpha amylase inhibition (Kamala et al. 2018; Tran et al. 2014; Udogadi and Onyenibe 2019).

### Table 6 Effect of Cyperus iria aqueous-ethanol extract on renal profile of streptozotocin-induced diabetic rats

| Parameter | Negative control group | Positive control group | Diabetic control group | CI 125mg/kg | CI 250mg/kg | CI 500mg/kg |
|-----------|------------------------|------------------------|------------------------|------------|------------|------------|
| Urea      | 31.6±0.92              | 38±0.44                | 49±0.54                | 50.2±0.86  | 44.8±0.66** | 41±0.63*** |
| Creatinine| 0.454±0.015            | 0.676±0.016            | 0.968±0.01             | 0.898      | 0.824±0.01** | 0.68±0.02*** |

Values are expressed as Mean ± SD (n=5)
*Shows *p < 0.05, **p<0.01, ***p<0.001 in comparison to diabetic control group DCG
OGTT indicated a decrease in area under curve exhibiting a decrease in blood glucose level. The delay in the apex glucose level reflects a change in absorption process. This change in absorption is linked with decreased expression of sodium glucose transport (SGLT-1). It suggests that a mechanism other than glycoside hydrolases is also involved. Results are in accordance with previous studies where hypoglycemia in baseline conditions is found to be associated with closing of potassium calcium channels in pancreatic β-cells (Malik et al. 2020). The higher antihyperglycemic activity of aqueous-ethanol extract of Cyperus iria might implicate the higher utilization of glucose. Similar antihyperglycemic activity has been reported earlier in similar genus (Bajpay et al. 2018).

A dose-dependent decrease in glucose was exhibited in STZ-induced diabetic rats by the aqueous-ethanol extract of Cyperus iria. STZ administration to rats resulted in hyperglycemia and hypoinsulinemia because STZ exerts cytotoxic effect on insulin secreting cells of pancreas (Amer et al. 2021). In the present study, antihyperglycemic activity was assessed by estimating blood glucose levels on day 0, 3, 6, 9, 12, and 15 after administration of aqueous-ethanol extract of Cyperus iria (125, 250, and 500 mg/kg) to diabetic rat. The raise in blood glucose level after STZ administration suggests a decrease in conversion of glucose into glycogen in diabetic control rats. Present study exhibited a positive correlation with an earlier investigation where it is claimed that flavonoids from Cyperus laevigatus showed antidiabetic effect and increased insulin serum level in diabetic rats (Elshamy et al. 2017). In the present study, the reduction in glucose level is attributed to the detected myrecetin and ferulic acid. Myrecetin possesses the ability to alter phosphorylation of insulin receptor, subsequently effecting glucose transporters subtype 4 (GLUT-4) translocation (Li and Ding 2012). It also protects the β-cells against high glucose-induced apoptosis by inhibition of endoplasmic stress (Gupta et al. 2020). Similarly, ferulic acid can scavenge superoxide anion radical effectively inhibiting the lipid peroxidation. Hydroxy and phenoxy groups of ferulic acid can donate electrons for the quenching of free radicals (Srinivasan et al. 2007). So it can be suggested that myrecetin and ferulic acid present in the aqueous-ethanol extract of Cyperus iria pose some roles in attenuating STZ-induced DM.

Acute oral toxicity exhibited the safety of aqueous-ethanol extract of Cyperus iria up to 2000mg/kg BW. It was observed that up to the dose of 2000 mg/kg inflammation, necrosis, fibrosis, and steatosis exhibited no significant difference with the normal control group; however, at the dose of 5000 mg/kg, significant difference in inflammation (p<0.001), necrosis, fibrosis, and steatosis (p<0.05) was observed. Likewise, inflammation, degeneration of acinar, and β-cells in the pancreatic tissues exhibited significant difference at the dose of 5000mg/kg. Although no mortality was observed in the rats up to 24 h but at high doses, some histological alterations resulted. Previously, no data is available on the acute toxicity study of aqueous-ethanol extract of Cyperus iria to the best of our knowledge. However, the genus as a whole was regarded as safe (Al-Hazmi et al. 2018; Khojaste et al. 2018).

Production of cytokines and pro-inflammatory markers has been associated with hyperglycemia. Advanced glycation products accumulation during hyperglycemia is associated with inflammation production. TNF-α and IL-6 are also proposed to increase the intensity and frequency of diabetic complication. Expressions of pro-inflammatory cytokines such as IL-6 and TNF-α are raised during hyperglycemia-induced injury (Malik et al. 2020). Present study demonstrated that aqueous-ethanol extract of Cyperus iria reduces the production of cytokines in a dose-dependent manner. The anti-inflammatory action of flavonoids and phenolics present in aqueous-ethanol extract of Cyperus iria is mainly due to its ability to inhibit the formation of pro-inflammatory mediators (e.g., adhesion molecules, cytokines, eicosanoids, and C-reactive protein) (Yahfoufi et al. 2018). Thus, the attenuation of pro-inflammatory cytokines and lipid peroxidation along with diminution of hyperglycemia and improved antioxidants by Cyperus iria treatment is clearly suggestive of its beneficial effects in DM. The results are in accordance with a previous claim where Cyperus rotundus, another species of the same genus, were claimed to possess anti-inflammatory potential by decreasing the plasma levels of TNF-α and adiponectin (Hussein et al. 2020).

Diabetes mellitus is associated with diabetic nephropathy leading to renal morbidity and mortality. In this condition, glomerular sclerosis and fibrosis occur causing loss of proteins in urine. Decreased insulin concentrations lead to minimize energy obtained from carbohydrates and increase catabolism of proteins leading to dysfunction of glomerulus (Malik et al. 2020). Decrease in urea and creatinine values was observed in animals after treatment with Cyperus iria aqueous-ethanol extract. Improvement in the renal parameters upon treatment is also supported by the histopathological findings where treatment with aqueous-ethanol extract of Cyperus iria resulted in improvement of tubular degeneration and glomerular thickening.

DM is associated with increased morbidity and mortality resulting from cardiovascular disease (CVD) (Lamacchia and Sorrentino 2021). Lipid metabolism is altered in diabetes. The lipid triad (elevated triglyceride, LDL-cholesterol levels, and decreased HDL-cholesterol concentrations) is considered an established risk factor for atherosclerosis in DM. The control of lipid levels is also important along with the reduction of plasma levels of TNF-α and adiponectin (Hussein et al. 2020).
levels and cardiovascular disease (Udogadi and Onyenibe 2019). Results are in concurrence with previous findings where phenols and flavonoids in plant extracts have been found to be associated with reduced lipid profiles exhibiting hypolipidemic effect (Zafar et al. 2020). Similarly, in another study, higher doses of hydroalcoholic extract of *Cyperus scariosus* showed increase in HDL levels and significant reduction in cholesterol, triglycerides, LDL, vLDL, and atherogenic coefficient level (Chawda et al. 2014).

High levels of ALT, AST and ALP in diabetic rats can be correlated with elevated transaminases in diabetic patients. The significant reduction in measures of ALT, AST, and ALP in STZ-induced diabetic rats was noted in the present study. Current investigation is in accordance with the previous study where *Cyperus rotundus* rhizomes, when given to diabetic animals, also lowered the measures of all hepatic enzymes (Singh et al. 2015). The improvement in the hepatic enzyme levels can be attributed to the decrease in free fatty acid, peroxides in the serum, and reduced oxidation (Harris 2005).

Antioxidant enzymes like SOD and GSH-ST are considered primary line of antioxidant defense. Oxidative stress is involved in the pathogenesis of type 2 DM. Free radicals either increase insulin resistance or impair insulin secretion. Numerous studies reported the possible contribution of free radicals and decrease antioxidant capacity with the induction of diabetes mellitus (El-Missiry et al. 2015; Udogadi and Onyenibe 2019). Present study indicated that treatment of STZ-induced diabetic rats with *Cyperus iria* aqueous-ethanol extract at 500 mg/kg dose expedite the activities of SOD and GSH-ST in plasma. In conformation with this report, the result of this study demonstrates a dose-dependent improvement in the activities of both markers, which is comparable to the standard treated group. Presence of phenolic acids and flavonoids in the plant extract might be responsible for improving oxidative stress because myrecetin, quercetin, ferulic acid, and kaempferol increase the activity of GST and SOD. The findings are in in affirmation with the previous report of Udogadi and Onyenibe (2019) where *Cyperus esculentus* treatment improved the levels of SOD, CAT, GSH, GPx as compared to control groups in STZ-induced diabetic rats. Myrecetin possess the potential to enhance the GST activity that catalyzes the conjugation of glutathione (GSH) to a wide variety of endogenous and exogenous electrophilic compounds. Similarly, SOD catalyzes the dismutation of superoxide anion (O2 radical) into hydrogen peroxide (H2O2), which is then degraded to H2O by CAT (Kandasamy and Ashokkumar 2013). Ferulic acid decreases the cell redox irregularities, kaempferol prevents oxidative damage by inhibiting lipid peroxidation (Al-Numair et al. 2015), and quercetin was found to be effective in controlling postprandial blood glucose in STZ-induced rats owing to reduction in the oxidative stress (Alam et al. 2014).

Histological examination of the pancreas, liver, and kidney of the control and extract treated rats indicated normal architecture. The hepatic scoring revealed a marked improvement in inflammation, necrosis, fibrosis, and steatosis (p<0.001) when compared to disease control group. Hepatic steatosis in diabetic rats occurred due to abnormal lipid deposition in hepatocytes caused by disordered lipid metabolism. Aqueous-ethanol *Cyperus iria* extract attenuated liver steatosis in type 2 DM, an effect that may be related to its capacity to diminish dyslipidemia. TG levels were also restored with aqueous-ethanol *Cyperus iria* extract treatment. Lipid overload in liver cells exacerbates oxidative stress, which promotes inflammation (Abdelmageed et al. 2021). Current scoring corroborates the abovementioned facts. Similarly, renal scoring of diabetic rats exhibited higher inflammation glomerular thickening and tubular degeneration scores. The treatment exhibited a significant improvement (p<0.001) in the renal scores of aqueous-ethanol *Cyperus iria* extract treated rats when compared to disease control group. The islets of Langerhans found in the pancreatic tissue were round in shape with normal cell lining. The results were consistent with Elshamy et al. (2017) that found that β-cells comprise the major of islets’ cells of rat’s pancreas. In the diabetic rats, the sections revealed a reduced pancreatic β-cell numbers compared to the control group. STZ-induced diabetes may be due to the selective destroying of pancreatic β-cells, which is responsible for the insulin production from endocrine cells. The pancreas of the diabetic rats treated with aqueous-ethanol *Cyperus iria* extract showed dramatic suppression of all abnormal histological changes as compared to the diabetic group. Regarding the mechanism by which aqueous-ethanol *Cyperus iria* extract can improve β-cells, researchers found that flavonoids, flavonoid glycosides, and phenolic acids exhibited a strong contribution as antioxidant agents that can regenerate the changes in the morphology of β-cells (Elshamy et al. 2017). Furthermore, the degenerative changes in pancreatic cells are associated with elevation in glucose, lipids, and inflammatory cytokines leading to induction of oxidative stress (Taha et al. 2018). These alterations result in inflammation, inhibition of acinar, and β-cell proliferation. Thus, in the current study, *Cyperus iria* aqueous-ethanol extract could protect β-cell functions and mass by suppressing oxidative stress as is evident from the histopathological scoring and also observed via improvement of glycemic and lipid metabolism.

**Conclusion**

The result of the present study revealed that aqueous-ethanol extract of *Cyperus iria*, significantly aided weight reduction, inhibited α-amylase and improved the lipid profile status. Aqueous-ethanol extract of *Cyperus iria* reduces the occurrence of oxidative stress and improves hyperglycemia owing
to its pharmacological properties in streptozotocin-induced type 2 diabetic rats. Therefore, the current study suggests that aqueous-ethanol extract of *Cyperus iria* may be recommended as a complementary and alternative supplement in type 2 diabetes after well-controlled clinical trials.

**Limitations of the study**

Some information about the experiment like number of calories in feed and urinary output is lacking in the current study. Although present study demonstrated the aqueous-ethanol extract of *Cyperus iria* as an ideal candidate to manage type 2 DM but some more mechanistic parameters along with immunohistochemistry are suggested as future prospects.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s11356-021-15917-9.

**Availability of data and materials** All data generated or analyzed during this study are included in this published article as supplementary files.

**Author contribution** M.S. performed the experimental work and participated in data interpretation. B.A. and F.M. participated in the data analysis, statistical analysis; A.S., M.M., and S.U. conceived the study, carried out the experimental design and data interpretation, and prepared and revised the manuscript. All authors read and approved the final manuscript.

**Ethics approval and consent to participate** The study was conducted followed by the approval of Animal Ethical Committee (IREC-2019-87) in accordance with the NC3Rs ARRIVE Guidelines, adhere to ethical guidelines of The Basel Declaration, the International Council for Laboratory Animal Science (ICLAS) ethical guidelines, and Directive 2010/63/EU. All procedures followed were in accordance with the standards set forth in the eighth edition of “Guide for the Care and Use of Laboratory Animals” (grants.nih.gov/grants/olaw/guide-for-the-care-and-use-of-laboratory-animals_prepub.pdf) published by the National Academy of Sciences, The National Academies Press, Washington, D.C.).

**Consent for publication** Not applicable

**Competing interests** The authors declare no competing interests.

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