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RESEARCH

Assessment of antihyperglycemic and antioxidant vigor of wheatgrass in streptozotocin-induced hyperglycemic rats

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Abstract

The present research evaluates in vivo antihyperglycemic together with antioxidant vigor of wheatgrass on streptozotocin-induced hyperglycemic rats. The previous research on in vitro activity of wheatgrass documented its immense potentiality so the current research was inspired to explore its in vivo efficacy. Subsequently, the experimental rats were arbitrarily segregated comprising of four groups of three animals each. A single intraperitoneal injection of streptozotocin was administered to the rats to induce hyperglycemia. Freshly prepared 0.1 M ice-cold citrate buffer was used at a dosage of 55 mg/kg body mass. However, administering wheatgrass extract to hyperglycemic rats caused a drop in glycemia which indicates antidiabetic potentials of wheatgrass. Evaluation of antioxidant activity in all the rat groups by DPPH and FRAP assay revealed that wheatgrass extract improved the antioxidant activities in hyperglycemic rats, but the antioxidant activities considerably declined in streptozotocin-treated rats versus normal control rats. Further, administration of STZ significantly reduced GSH levels in hyperglycemic rats in comparison with control rats but subsequently the supplementation with wheatgrass extract considerably elevated GSH content in hyperglycemic rats. Moreover, MDA content considerably declined in hyperglycemic rats when treated with wheatgrass extract in contrast to hyperglycemic control rats. The supplementation of wheatgrass extract in hyperglycemic rats restored MDA content near to normal control rats. A considerable elevation in sialic acid of plasma was recorded in STZ-induced hyperglycemic rats versus control. However, supplementing extract of wheatgrass to diabetic rats restored the content of sialic acid. Subsequently, administration of wheatgrass extract to hyperglycemic rats showed a considerable decline in lipid profile, viz., total cholesterol, triglycerides, LDL, and VLDL levels, and a rise in HDL level. Furthermore, wheatgrass extract supplementation to hyperglycemic rats documented a significant decline in liver function parameters such as SGOT, SGPT, and alkaline phosphatase levels, as well as kidney function parameters such as urea and creatinine levels. Finally, the HPLC of wheatgrass extract revealed the presence of chlorophyllin and rutin conferring antidiabetic and antioxidant efficacy.

Keywords Antidiabetic · Antioxidant · Streptozotocin · Hyperglycemic rat · Wheatgrass

Introduction

Wheatgrass (*Triticum aestivum*) has received widespread attention worldwide with great medicinal as well as nutritional potential. *Triticum aestivum* is the juvenile grass of wheat grains and belongs to the Poaceae family (Fig. 1). *Triticum aestivum* is widely known as "living food" owing to its high nutritional content, comprising diverse minerals, viz., molybdenum, sodium, potassium, aluminium, selenium, sulphur, copper, iron, zinc, iodine, phosphorus, calcium, magnesium, and boron together with vitamin A, B, C, E, and K along with enzymes, viz., lipase, cytochrome oxidase, superoxide dismutase, amylase, transhydrogenase, and protease, as well as varied amino acids (Walters 1992; Hanninen et al. 1999; Kulkarni et al. 2006; Swati et al. 2010; Mishra et al. 2025). *Triticum aestivum* has tremendous pharmacological effects, viz., antimicrobial, antidiabetic, antioxidant, antiulcer, wound healing, antiaging, diuretic, immunomodulatory, anticancer, and antiarthritic (Roshan et al. 2016; Tripathi et al. 2026).

Diabetes is an alarming lifestyle disorder globally, marked by elevated postprandial and fasting blood sugar levels (Boyle et al. 2001). Diabetes is caused by either insulin deficiency or insulin malfunction. Type I diabetes is characterized by the deficiency of insulin. As a result, persons with this condition require exogenous intake of

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Fig. 1 Pictorial presentation of wheatgrass

insulin, whereas persons with Type II diabetes do not respond to insulin. Type II diabetes is perhaps most prevalent, accounting for 90% of diabetic population (Pethe et al. 2024). The WHO has documented hyperglycemic cases as in 2010 to be around 285 million globally, and the figure is expected to climb to about 438 million by 2030 (Snehalatha and Ramachandran 2009). Wheatgrass' antihyperglycemic action derives mostly from its capacity to improve pancreatic tissue function by increasing insulin production or reducing glucose absorption in the intestine. Moreover, bioactive compounds from several herbs are repeatedly documented to have antihyperglycemic efficacy (Malviya et al. 2010).

Reactive oxygen species (ROS) are perpetually generated in living beings owing to numerous metabolic activities and exposure to various physicochemical factors. The higher concentrations of ROS are harmful and can destroy important biological components, resulting in disruption of redox signaling. Furthermore, oxidative damage results in the onset of diabetes-related problems. In hyperglycemic conditions, ROS are continuously generated and data suggest that diabetes causes alterations in the level of antioxidant enzymes in diverse organs. However, antioxidants are vital in shielding the body against oxidative damage (Day 1998, Eurich et al. 2007, Ahmed 2005). As a result, a medication with antioxidant as well as antidiabetic properties could potentially be beneficial against diabetes. Along with carotenoids, tocopherols, flavonoids, lignans, phenolic acids, and alkylresorcinols, wheatgrass also contains several flavonoids (Moshawih et al. 2022). Literature review reveals that very confined clinical data and in vivo investigation were documented on antihyperglycemic and antioxidant vigor of wheatgrass. Therefore, the current research investigation was envisaged to assess the aforementioned prospect of wheatgrass.

Materials and methods

Sowing of wheat grains

Superior quality wheat grains (Sharbati cultivar) were procured from Prayagraj (U.P.), India. The experiment entailed growing wheatgrass indoors (Fig. 2). The clay pot was packed with three inches, which consisted of three parts soil and one part compost. Wheat grains that had been soaked overnight were then equally placed over it, followed by a half inch of humus, and little amount of water was sprayed uniformly over it. Subsequently, 3–4 h of partial sunlight was given per day to stimulate wheatgrass development and prevent excessive nutrient loss from direct sunlight exposure. Moreover, the soil pH was slightly acidic to neutral (6.0–7.0) and the relative humidity of 40–60% was maintained for the optimal cultivation of wheatgrass. The seeded wheat grains began to grow, and the wheatgrass was harvested at 7th day; it was nearly 6–9 inches in length (Jain and Argal 2014). After harvesting the wheatgrass, the herbarium was prepared for its identification and deposited in the Department of Family and Community Sciences, University of Allahabad, Prayagraj, 211002, Uttar Pradesh, India.

Extraction of wheatgrass

The wheat sprouts were soaked, rinsed, drained, and dried at 55 ± 2 °C for 6 h. Dried wheatgrass was powdered by a mechanical grinder and passed through a sieve. The wheatgrass-powdered sample was extracted by cold maceration in a beaker, and absolute ethanol was mixed in a ratio of 1:20 (1 g powder to 20 ml solvent), kept at room temperature for up to 48 h, and filtered via Whatman No. 1, and the filtrate was then reduced to one-tenth and lyophilized until the solvent completely dried (Dholi 2018).

Animal care and acclimatization

Male experimental rats with body weight of 150 ± 15 g and 4.5 ± 0.5 months old were purchased from Allahabad, Uttar Pradesh, India. The rats were accommodated in an environment of light–dark cycle (12 h) at 25 ± 5 °C (Fig. 3). These rats are adapted for 7 days under the aforementioned conditions before commencing experiments. The experimental rats were sustained on standard nutrient-dense pellets having complete calories

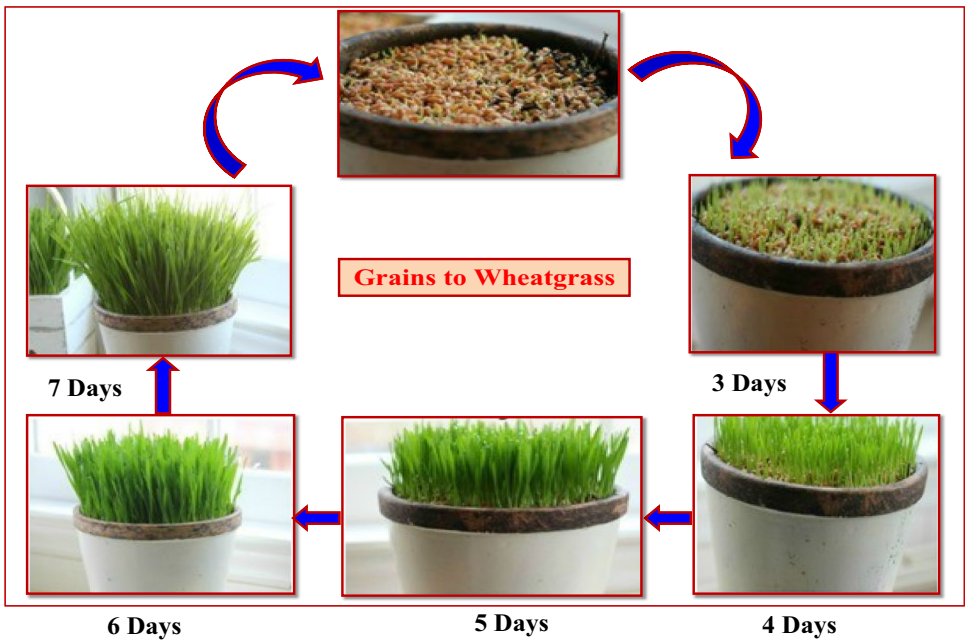

Fig. 2 Pictorial presentation of wheatgrass cultivation

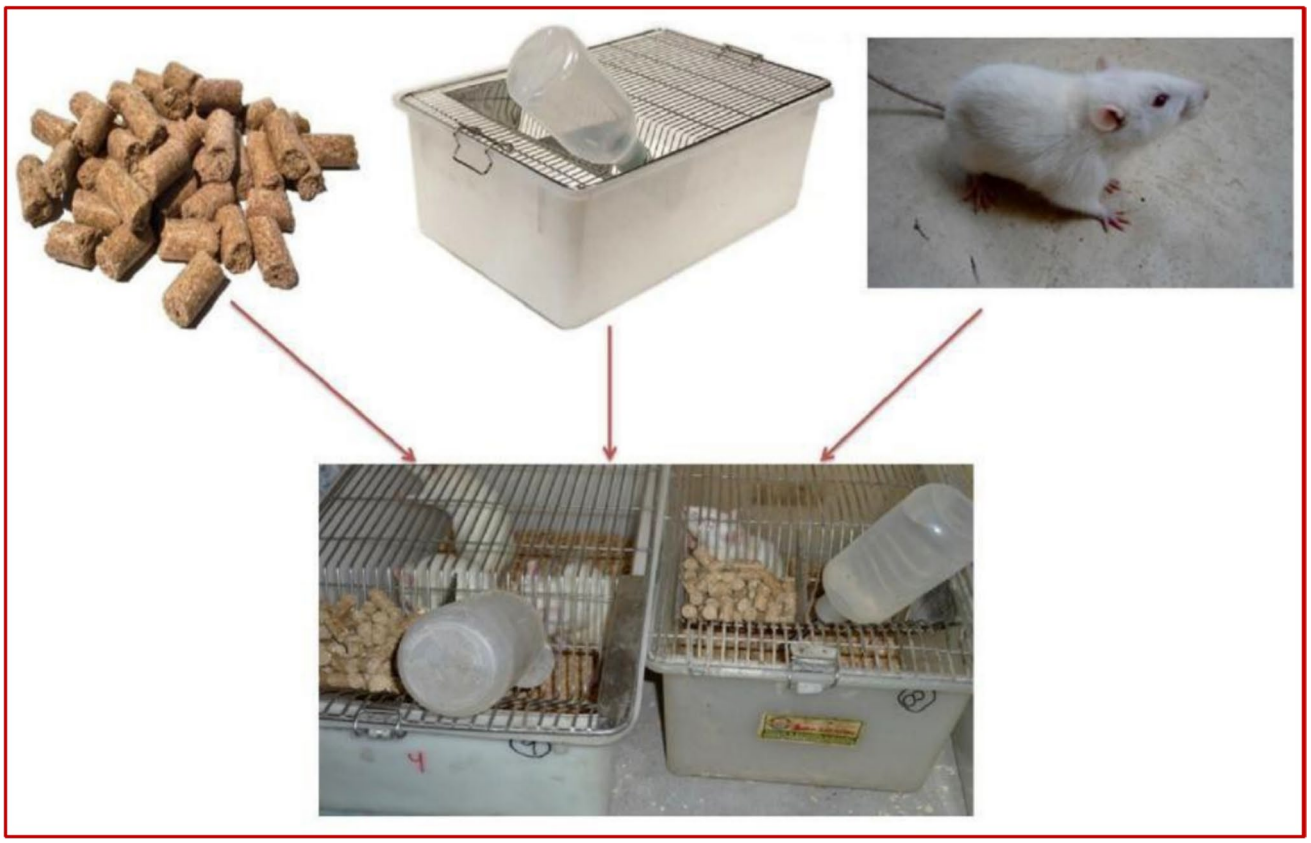

Fig. 3 Model experimental rat

along with drinking water. The protocols employed followed the guidelines for laboratory animals and were endorsed by IEC, University of Allahabad, Uttar Pradesh, India.

Chemically induced diabetes

A single intraperitoneal injection of streptozotocin was administered to the rats under investigation to elicit hyperglycemia. Freshly prepared 0.1 M ice-cold citrate buffer at a dosage of 55 mg/kg body mass was used (Ghasemi and Jeddi 2023; Islam and Wilson 2012). Experimentally, streptozotocin is able to induce type I diabetes, which destroys pancreatic β-cells. Further, to prevent hypoglycemia, rats were kept in their cage with 10% glucose solution bottles for 24 h. Eventually, in a week, diabetes in rats was ascertained by a fasting blood sugar level of > 250 mg/dl using a glucometer (Gluco care™ ultima). The diabetic rats were used for the subsequent research.

Model animal and research protocol

Present research focus to explore the effect of wheatgrass on streptozotocin-induced diabetic rats. The experimental rats were arbitrarily segregated comprising of four groups of three animals each (Tripathi et al. 2021; Kamath et al. 2023):

- Category I: Normal control
- Category II: Normal treated with wheatgrass extract 100 mg/kg body weight
- Category III: Diabetic control, injected a single dose intraperitoneally with STZ
- Category IV: Diabetic treated with wheatgrass extract 100 mg/kg body weight, using gavage technique after STZ injection and supplementation of extract continued up to 30 days

Finally, the experimental rats were sacrificed using mild anesthesia after being fasted for 12 h. After cardiac puncture, the blood sample was collected into heparin-rinsed anticoagulant syringes containing 10 unit/ml. Subsequently, the blood sample was centrifuged (1500×*g* for 10 min) to obtain plasma.

and stored at 4 °C. After removing the plasma buffy coat, uppermost 15% packed RBCs, red blood cells were washed two times with chilled phosphate-buffered saline.

Antioxidant potential of wheatgrass

DPPH assay

To 2 ml, 0.1 mM DPPH with methanol and 1.9 ml, 10 mM phosphate buffer, 100 µl of plasma was added along with 2 ml, 10 mM phosphate buffer and equal quantity DPPH solution as control. The above sample was kept at 21 °C for 30 min, subsequently centrifuged (5 min) at 1000×*g*. Finally, the absorbance was recorded at 517 nm, using a blank as methanol. Values for the plasma (A) and control (A0) were compared; the formula 100 (A0 − A)/A0 was used to estimate the percentage of radical scavenging efficacy (Szabo et al. 2007).

FRAP Assay

In plasma samples, the total antioxidant activity was assessed with the modified FRAP method (Benzie and Strain 1996). The reagent was made from 20 mmol/l ferric chloride, 300 mmol/l acetate buffer, and pH of 3.6 along with 10 mmol/l of 2,4,6-tripyridyl-s-triazine in 40 mmol/l HCl in a 10:1:1 ratio. To 100 µL of plasma, the reagent (3 ml) was added and thoroughly stirred. Finally, the absorbance was recorded at 593 nm in 30-s intervals up to 4 min. Calibration was performed by an aqueous solution with Fe (II) concentrations (100 to 1000 µmol/l). A regression equation based on plasma FRAP values (µmol Fe (II)/L) was employed for computation.

Glutathione (GSH) assay

Glutathione (GSH) was estimated following Beutler (1984). The SH group reduces 5,5′-dithiobis-(2-nitrobenzoic acid), resulting in a yellow anionic product, and the optical density at 412 nm was recorded. Glutathione concentration is represented in mg/ml of packed red blood cells.

Sialic acid assay

Sialic acid was evaluated following Spyridaki and Siskos 1996. To 0.10 ml of periodic acid (0.04 M), 0.5 ml of plasma was added, mixed properly, and kept up to 30 min in an ice bath. Subsequently, 1.25 ml of $C_6H_4(OH)_2$ working solution (0.125 ml of 0.1 M $CuSO_4$ solution, 5 ml of 6.0% $C_6H_4(OH)_2$ solution together with 19.875 ml of distilled water, adjusted with 50 ml, 10 M HCl) was mixed and subjected to heating for 5 min at 98 °C. After cooling for 2 min, n-butanol (3.25 ml) was mixed, stirred thoroughly, and kept in water bath at 37 °C for 3 min to stabilize colour. Finally, using a reagent blank, the absorbance was recorded at 625 nm.

Biochemical analysis

The level of sugar in the blood was measured by an Accu-Check Active Glucometer (Roche Diagnostics, Mannheim, Germany). The serum lipid profile tests (total cholesterol, triglycerides, LDL, HDL, and VLDL), as well as liver function tests (SGOT, SGPT, and alkaline phosphatase) and kidney function tests (creatinine and urea levels), were performed using Erba Diagnostics reagent kits from Mannheim, Germany.

Identification of bioactives of wheatgrass by HPLC

Wheatgrass extract's bioactive compounds were analyzed using reversed-phase (C18) high-performance liquid chromatography (Systronics), C-18 column (HiQ Sil C18-HS 4.6 mm × 250 mm × 5 µl). p, and a column thermostat (Table 2). Wheatgrass ethanol extract was prepared using standard solutions and filtered by 0.22 µm Milli-pore (Billerica, USA).

Mobile phase, flow rate, and wavelength were all set to standard (Table 3). Twenty microliters of sample was injected into the column. The bioactive compounds present in wheatgrass extract were estimated by comparing the peak retention time and area of the wheatgrass extract chromatographic peak with that of standard solutions.

Data analysis

The data of experimental findings were analysed via ANOVA (one-way) with Tukey's test along with GraphPad Prism (Version 5.01) for Windows and by Design-Expert 7.0.0. All the p-values, viz., $p < 0.05$, $p < 0.01$, and $p < 0.001$, are analytically significant and all the values are indicated as mean ± SD.

Results and discussion

Body weight of experimental rats

In this investigation, the weight of streptozotocin-induced hyperglycemic rats was reduced, owing to the breakdown of tissue protein and fat, as well as a decreased glucose metabolism, which results in the unavailability of carbohydrates for energy, all of which contribute to body weight loss. Our findings corroborate with other researchers' investigations which documented that hyperglycemia is associated with weight loss (Rossmeisl et al. 2003; Diego et al. 2019). In this investigation, the weight of STZ-induced hyperglycemic rats declined. However, oral supplementation with wheatgrass extract improved the weight of hyperglycemic rats compared to streptozotocin-induced hyperglycemic control rats (Fig. 4).

Body glucose of experimental rats

Streptozotocin (STZ) causes diabetes by destroying β-cells of the pancreas, leading to the decreased insulin release and ultimately hyperglycemia (Gilman et al. 1990). Many evidences from various experiments have demonstrated that the bioactive compounds from medicinal plants reduce the risk of hyperglycemia by lowering glucose levels (Robertson et al. 1992). Figure 5 depicts the changes in blood glucose levels among different groups of rats under investigation. Diabetic control rats had considerably elevated blood glucose levels compared to normal rats. However, supplementation of wheatgrass extract notably reduced the glycemia level in hyperglycemic rats under study. Finally, it was recorded that administration of wheatgrass extract marked a considerable decline in glycemia level in hyperglycemic rats and a backslide to normal level, via increasing the efficacy of liver hexokinase, which helps in promoting glycolysis and thereby increasing the glucose utilization (Chude et al. 2001). Wheatgrass extract administration may increase insulin release from β-cells, causing a substantial decline in glucose levels (Mohan et al. 2013). Several scientific investigations have already demonstrated and documented that phenolic chemicals and flavonoids have antidiabetic properties (Imran et al. 2010); their antidiabetic potentiality to regenerate β-cells of the pancreas is well documented (Chika and Bello 2010; Suba et al. 2004). The findings of the research strongly demonstrate the antidiabetic potentiality of wheatgrass.

Antioxidant potential of wheatgrass

DPPH assay

DPPH method is used to screen antioxidant compounds (Soler-Rivas et al. 2000). Antioxidants scavenge oxidized DPPH radicals, resulting in stable molecules of reduced DPPH. These findings demonstrate that wheatgrass extracts contain a potent inhibitor that can serve as a primary antioxidant. The more is the inhibiting potential, the greater is the antioxidant activity (Siddhuraju et al. 2002). STZ administration significantly reduced radical scavenging activity ($p < 0.001$). Furthermore, supplementation of wheatgrass extract considerably increased radical scavenging activity (Fig. 6).

FRAP assay

Antioxidants act by eliminating or leading to their transformation of free radicals into less toxic compounds

for cells (Sies 1993). Hyperglycemia in diabetes promotes increased oxidative stress, which leads to the consequences of diabetes mellitus due to decreased antioxidant enzymes, increased free radicals, and lipid peroxidation (Nishikawa et al. 2000) and also promotes insulin sensitivity and resistance. The antioxidant activity of all the groups under investigation was assessed using the FRAP test. Supplementation of wheatgrass extract enhanced antioxidant activity versus hyperglycemic rats. Moreover, streptozotocin significantly decreased antioxidant activity in comparison to normal rats (Fig. 7).

Glutathione (GSH) assay

The antioxidant defence system is altered by diabetes (Lim et al. 2010). Reduced glutathione (GSH) works as a free radical scavenger, providing protection against free radical-induced cellular damage. Previous published findings documented that GSH content declines in hyperglycemic rats (Jiang et al. 1990). These alterations may be attributed to the production of free radicals, glucose oxidation, and nitric oxide-donor properties of streptozotocin (Mohamed et al. 1999).

STZ-induced diabetic rats had elevated levels of reduced GSH as that of rats as control. The STZ-induced hyperglycemic rats had considerably lower content of reduced GSH versus that of rats as control. The dose of STZ substantially decreased GSH levels of hyperglycemic rats.

Administering the extract of wheatgrass significantly boosts GSH levels in hyperglycemic rats (Fig. 8). In streptozotocin-induced hyperglycemic rats, wheatgrass' ability to restore altered GSH levels demonstrates its antioxidant potential. Wheatgrass contains tannins, polyphenols, flavonoids, saponins, and sterols which have the ability to act as antioxidants and consequently regulate blood glucose levels in diabetes.

Malondialdehyde (MDA) assay

Hyperglycemia alters the lipid profile by rendering cells highly prone to oxidative degradation of lipids (Patricia 2009). Several research investigations have reported that polyunsaturated fatty acids of cell membrane are more prone to attack by free radicals because of the presence of multiple bonds (Butterfiel et al., 1998). Increased lipid peroxidation, a key indicator of oxidative stress, impairs membrane function by reducing membrane strength. The lipid peroxidation generates malondialdehyde (MDA) which can be employed to quantify lipid peroxides by its reaction with thiobarbituric acid (Esterbauer et al. 1991).

Streptozotocin-induced hyperglycemic rats had considerably greater erythrocyte MDA levels versus normal rats as controls. Furthermore, supplementation with extract

Table 1 Specification for HPLC analysis

HPLC instrument	Systronics
Column	HiQ Sil C18-HS
Pump	Isocratic type
Column size	4.6 mm × 250 mm × 5 µm
Injection volume	20 µl
Run time	20 min
Concentration of extract	10 µl/ml (dissolved in ethanol)
Column temperature	25 °C
Determinant	Chlorophyllin, rutin

Table 2 Mobile phase, flow rate, and wavelength of standards used in HPLC analysis

Standards	Mobile phase	Flow rate	Wavelength
Chlorophyllin	Methanol	1.0 ml/min	423 nm
Rutin	Methanol: 0.4% H_3PO_4	1.0 ml/min	220 nm

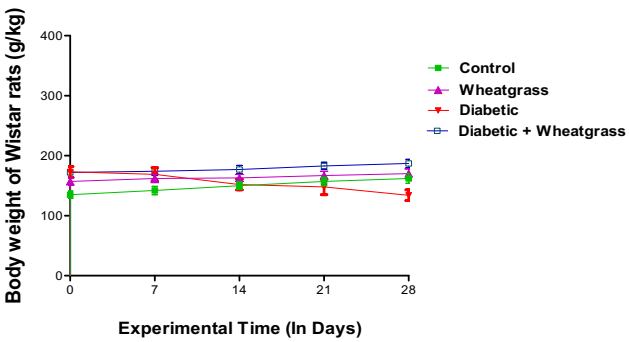

Fig. 4 Effect of wheatgrass extract on streptozotocin (STZ)-induced diabetes on the weight of experimental rats in a 28-day period. Body weight is expressed in grams. Values represent mean ± SD

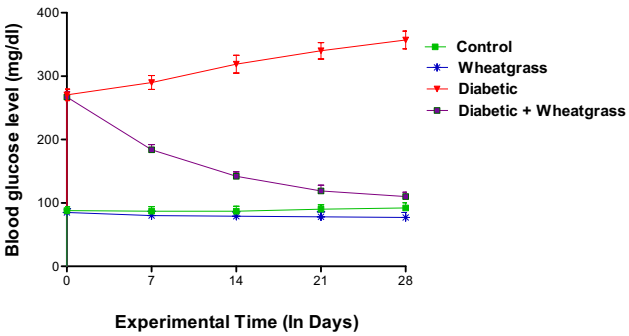

Fig. 5 Effect of wheatgrass extract on streptozotocin (STZ)-induced diabetes on glycemia level in tested rats. Concentration of blood glucose is expressed as mg/dl. Values represent mean ± SD. Blood glucose levels in the experimental rats are taken after the 0, 7, 14, 21, and 28 days of supplementation

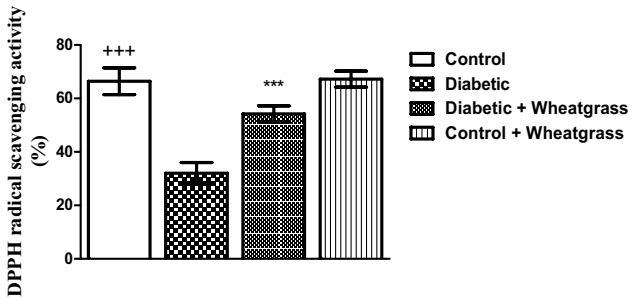

Fig. 6 Effect of wheatgrass extract on streptozotocin (STZ)-induced diabetes on DPPH activity of plasma in tested rats. Values are presented as mean ± SD. + + + $p < 0.001$, *** $p < 0.001$ compared to diabetic

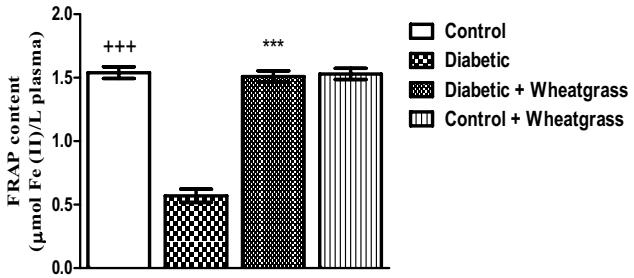

Fig. 7 Effect of wheatgrass extract on streptozotocin (STZ)-induced diabetes on total antioxidant potentiality of plasma in tested rats. FRAP value is expressed in µmol Fe(II)/L plasma. Values are presented as means ± SD. + + + $p < 0.001$, *** $p < 0.001$ compared to diabetic

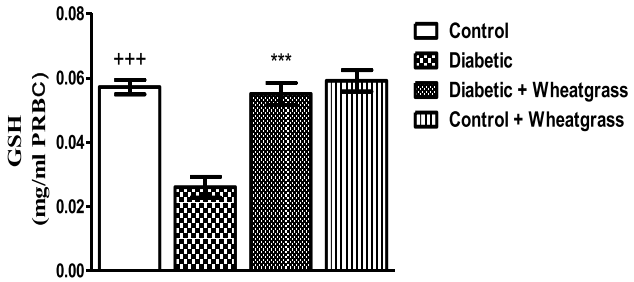

Fig. 8 Effect of wheatgrass extract on streptozotocin (STZ)-induced diabetes on the level of GSH in experimental rats. GSH concentration is expressed as mg/ml PCRB. Values are presented as mean ± SD. + + + $p < 0.001$, *** $p < 0.001$ compared to diabetic

of wheatgrass to streptozotocin-induced hyperglycemic rats decreased the MDA levels significantly in contrast to diabetic rats as controls (Fig. 9). Hyperglycemic rats administered with the extract of wheatgrass restored MDA levels close to normal control rats and prevented the markers causing oxidative degradation of lipids from reverting to usual levels. Our results align with earlier reports that

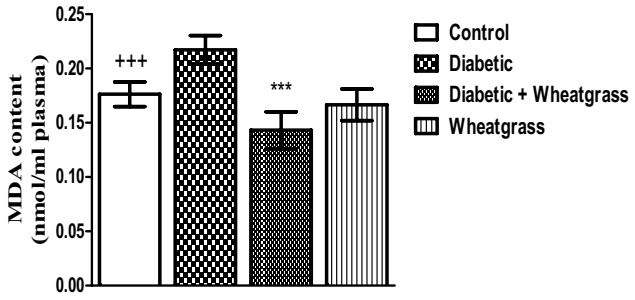

Fig. 9 Effect of wheatgrass extract on streptozotocin (STZ)-induced diabetes on malondialdehyde (MDA) level in experimental rats. MDA level is represented as nmol/ml. Values are expressed as mean ± SD. + + + $p < 0.001$, *** $p < 0.001$ compared to diabetic

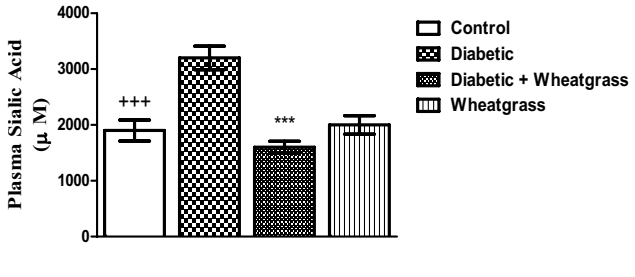

Fig. 10 Effect of wheatgrass extract on streptozotocin (STZ)-induced diabetes on the content of sialic acid in experimental rats. Concentration of plasma sialic acid is represented as µmol. Values are represented as mean ± SD. + + + $p < 0.001$, *** $p < 0.001$ compared to diabetic

demonstrate wheatgrass extract has high antioxidant activity (Durairaj et al. 2014; Kulkarni et al. 2006).

Sialic acid assay

The diabetic rats demonstrated considerably elevated plasma sialic acid compared to controls; the above findings are in line with Gorgun et al. (2002). The elevated sialic acid levels in streptozotocin-induced hyperglycemic rats can be due to increased production of sialic acid in insulin-independent tissues and greater sialyltransferase enzymatic activity (Sonmez et al. 1997). Moreover, oral administration of wheatgrass extract to diabetic rats restored sialic acid levels to near normal (Fig. 10).

Lipid profile test

Several research have documented that significant variation in lipid profile results in hyperglycemia (Aissaoui et al. 2011; Li et al. 2012). Furthermore, Keenoy et al. (2005) and Ravi et al. (2005) have documented that aberration in lipid metabolism altogether leads to enhancement of serum lipid levels and lipoproteins. Moreover, insulin plays a vital role in lipid metabolism by triggering the action of

Table 3 Effects of wheatgrass on lipid profile of steptozotocin-induced diabetic rats

S. No	Parameters	Control	Wheatgrass	Diabetic	Diabetic + wheatgrass
1	Total cholesterol (mg/dl)	67.9 ± 6	63.2 ± 7	104.7 ± 7***[a]	83.9 ± 7***[b]
2	Triglyceride (mg/dl)	74.6 ± 6	76.3 ± 7	157 ± 15***[a]	120.5 ± 7***[b]
3	LDL (mg/dl)	25.6 ± 3	24.1 ± 3	52.2 ± 3***[a]	38.6 ± 4***[b]
4	VLDL (mg/dl)	16.1 ± 3	16.8 ± 3	31.8 ± 3***[a]	21.3 ± 4***[b]
5	HDL (mg/dl)	32.4 ± 4	30.6 ± 4	21.8 ± 3*[a]	32.9 ± 4***[b]

Values represent mean ± SD. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

[a]Diabetic compared to control

[b]Diabetic + wheatgrass compared to diabetic

Table 4 Effects of wheatgrass on liver and kidney function test of steptozotocin-induced diabetic rats

S. No	Parameters	Control	Wheatgrass	Diabetic	Diabetic + wheatgrass
1	SGOT (U/L)	110 ± 15	100.50 ± 14	160 ± 13***[a]	125 ± 13**[b]
2	SGPT (U/L)	5.59 ± 1.49	3.77 ± 0.92	19.3 ± 3.07***[a]	9.47 ± 2.21***[b]
3	Alkaline phosphatase (U/L)	257.56 ± 19.12	288 ± 24	525 ± 37.27***[a]	352 ± 30***[b]
4	Urea (mg/dl)	39.90 ± 4.38	44 ± 4.03	61.24 ± 7.34***[a]	50 ± 5.89*[b]
5	Creatinine (mg/dl)	0.394 ± 0.07	0.314 ± 0.03	0.488 ± 0.02**[a]	0.417 ± 0.02*[b]

Values represent mean ± SD. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

[a]Diabetic compared to control

[b]Diabetic + wheatgrass compared to diabetic

lipolytic hormones on peripheral fat which ultimately hydrolyses triglycerides and averts mobilization of free fatty acids, apart from regulation of carbohydrate metabolism. In hyperglycemic animals, increase in triglyceride, total cholesterol, VLDL, and LDL and decrease in HDL are on account of enhanced lipolysis in adipose tissue, and decline in the activity of insulin-dependent lipoprotein lipase resulted in enhanced level of fatty acids (Kavitha et al. 2016).

Table 1 describes the effect of wheatgrass extract on serum lipid profile, viz., total cholesterol (TC), triglyceride (TG), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), and high-density lipoprotein (HDL). Enhanced level of serum total cholesterol, triglyceride, LDL, and VLDL with decline in HDL levels was recorded in streptozotocin-induced hyperglycemic rats in contrast to normal control rats. Subsequently, the supplementation of wheatgrass extract to streptozotocin-induced hyperglycemic rats demonstrated a considerable decline in the serum triglycerides, total cholesterol, LDL, and VLDL levels, and a significant enhancement in the HDL cholesterol levels. Moreover, the rise in lipid profile in hyperglycemic rats is on account of insulin deficiency and glucose intolerance (Tchobroutsky 1978; Rodrigues et al. 1986). The level of HDL was demonstrated to be considerably high in hyperglycemic rats which proves that together with improved lipid profile by administration with herbal combinations, the increase in HDL level also limits atherosclerosis and coronary hazards (Patil et al. 2012).

Liver and kidney function test

Table 4 describes the effect of wheatgrass extract on liver and kidney function tests. Hyperglycemia leads to variation in liver function tests. The increased level of serum enzymes AST, ALT, and ALP in streptozotocin-induced hyperglycemic rats can be a preliminary sign of liver injury (Senthilkumar and Subramanian 2007), as the impairment in liver tissues liberates these enzymes into the bloodstream, leading to the increase in the activities of these enzymes. Liver injury leads to hepatotoxic effects of streptozotocin (Navarro et al. 1993). However, supplementation of wheatgrass extract rendered a considerable decline in SGOT, SGPT, and alkaline phosphatase levels. Moreover, supplementation of wheatgrass extract demonstrated a considerable decline in the activities of liver enzymes in streptozotocin-induced hyperglycemic rats.

Further, the hyperglycemia also damages the kidney and alters its functions, and glomerular filtration rate (GFR) is impaired in hyperglycemic conditions (Dabla et al., 2010). However, enhanced serum urea and creatinine levels are preliminary clinical parameters of kidney disease. In the present research investigation, a considerable enhancement in serum urea and creatinine levels was recorded in streptozotocin-induced hyperglycemic rats in contrast to

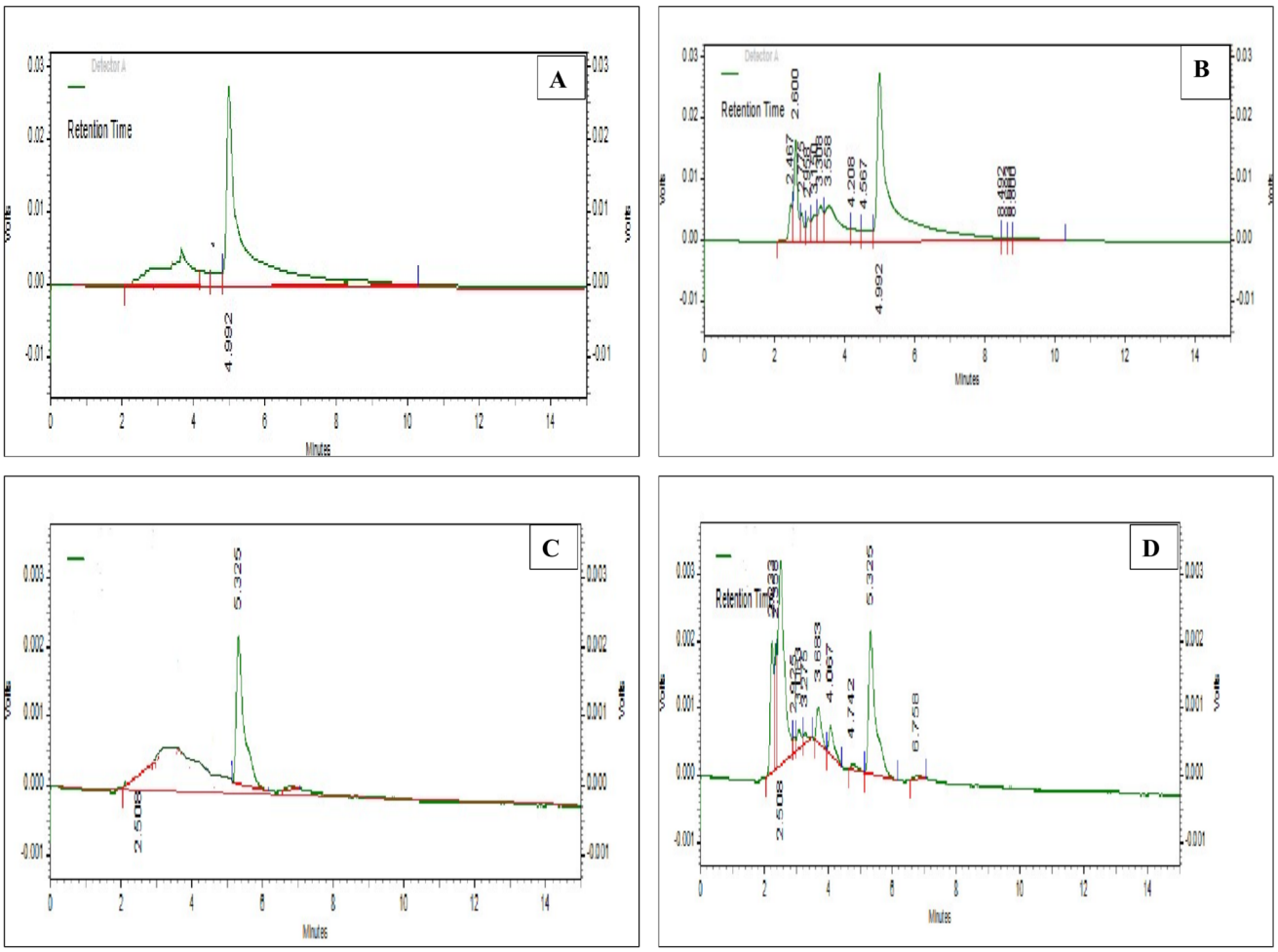

Fig. 11 High-performance liquid chromatography of ethanolic extract of wheatgrass: **A** standard chlorophyllin (423 nm), **B** wheatgrass extract (area % = 4.992), **C** standard rutin (220 nm), and **D** wheatgrass extract (area % = 5.325)

normal control rats. The results of this research corroborate with the findings of Zarei et al. 2015. Moreover, oral administrations of wheatgrass extract lead to a considerable decline in urea and creatinine levels.

Bioactives of wheatgrass by HPLC

Chromatographic analysis of the bioactive compounds found in wheatgrass was carried out using HPLC. In this research, two different phenolic standards were compared to the chromatograms produced by wheatgrass extract. Phenolic compounds exhibit a wide spectrum of antioxidant properties due to their scavenging ability via their hydroxyl groups (Uddin et al. 2014). Results of HPLC estimation demonstrated chlorophyllin and rutin as the bioactives in wheatgrass extract conferring enhanced antihyperglycemic and antioxidant activity. Similarly, the bioactives from herbs were also documented (Pandey and Gupta 2019).

Standard chlorophyllin was determined, with a characteristic peak at 4.992. Two characteristic peaks were seen at 423 nm in a chlorophyllin sample, with 4.992 having the highest area percentage. The standard rutin was determined, with a characteristic peak at 5.325.

Subsequently, the wheatgrass sample was analyzed at 220 nm. Eleven characteristic peaks were identified, with the largest area percentage of 5.325. The HPLC chromatogram of standards and wheatgrass extracts is presented in Fig. 11.

Conclusion

Current research investigation on antidiabetic and antioxidant efficacy was envisaged to evaluate wheatgrass extract on streptozotocin-induced hyperglycemic rats. Diabetes induces increased ROS production and disruption of redox signaling leading to diabetes mellitus. Results revealed that supplementation of wheatgrass extract to

hyperglycemic rats caused progressive reduction in glycemia in STZ-induced hyperglycemic rats which indicates antihyperglycemic potentials of the wheatgrass extract. The DPPH and FRAP assays showed that the treatment with wheatgrass extract improved the antioxidant activities in hyperglycemic rats. Further, the supplementation of wheatgrass extract significantly elevates GSH levels in hyperglycemic rats which indicates antioxidant potentials of the wheatgrass extract. Moreover, hyperglycemic rats administered with wheatgrass extract restored the MDA level near to normal control rats. Furthermore, the supplementation of wheatgrass extract to hyperglycemic rats restored the normal levels of sialic acid in plasma. The hyperglycemic rats supplemented with wheatgrass extract showed a considerable decline in the serum lipid profile, viz., total cholesterol, triglycerides, LDL, and VLDL levels, and a significant rise in the HDL cholesterol levels. Moreover, wheatgrass extract administration to hyperglycemic rats documented a significant decrease in the liver function parameters such as SGOT, SGPT, and alkaline phosphatase levels along with kidney function parameters such as urea and creatinine levels. Finally, the wheatgrass ethanolic extract showed the presence of chlorophyllin and rutin by the HPLC analysis. In a nutshell, wheatgrass possesses immense antidiabetic potential and confers the great efficacy to improve the antioxidant status and provide defence against oxidative stress. However, further efforts to explore new pharmacological potential of wheatgrass and its molecular mode of actions and also to develop new functional and nutraceutical food products will be the future direction of this research.

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Declarations

Conflict of interest The authors declare no competing interests.

Ethical approval This article does not contain any studies with human participants performed by any of the authors.

Informed consent For this type of study, informed consent is not required.

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