Clinical Phytoscience

ORIGINAL CONTRIBUTION

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Sorghum halepense (L.) Pers rhizomes inhibitory potential against diabetes and free radicals

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Abstract

Background: Owing to the side effects of synthetic medicine and less effectiveness against different syndromes, the researchers have focused on phytotherapy to overcome these problems. The purpose of this project was to study the in vitro phytochemical, cytotoxic, total phenolic, antioxidant and antidiabetic activities of the methanol extract of the rhizome of *Sorghum halepense* (L.) Pers and its *n*-hexane, chloroform and aqueous fractions. Thereafter, to conduct in vivo evaluation of the effective extract for its antidiabetic and antioxidant characteristics.

Methods: Cytotoxic, total phenolic content and antidiabetic properties were ascertained by brine shrimps lethality, Folin- Ciocalteu reagent and alpha-amylase inhibition assays respectively while antioxidant activities were investigated through DPPH, ABTS and H_2O_2 assays. The methanolic extract was assessed in vivo for its antidiabetic and antioxidant activities by using Wistar albino rats.

Results: The phytochemical investigation of the methanolic extract and its unlike fractions revealed the availability of alkaloids, cardiac glycosides, flavonoids, terpenes, steroids, carbohydrate and proteins while lack of saponins and gums in methanolic extract. Steroids and carbohydrates were only present in aqueous and chloroform fraction respectively while both fractions contained proteins and alkaloids. Cardiac glycosides and flavonoids were absent in aqueous and chloroform fractions respectively. The highest brine shrimps lethality (70.5 ± 1.2), total phenolic content (28.30 ± 1.3 mg GAE/g), free radicals scavenging potential i.e. DPPH (40.02%), ABTS (40.48%) and H_2O_2 (50.85%) and alpha amylase inhibition (61.87%) was shown by the methanolic extract. The in vivo results did not disclose any sign of acute toxicity. The diabetic control showed a noteworthy ($P < 0.05$) decline in weight, HDL and glutathione and a raised level of bilirubin, blood glucose, urea, creatinine, triglyceride, LDL, VLDL, ALT, ALP, AST, SOD, catalase. The mentioned alterations were restored considerably ($P < 0.05$) by treatment of diabetic rats with methanolic extract of *Sorghum halepense* (L.) Pers (150 and 300 mg/kg b.w.).

Conclusion: It is concluded that the extract of rhizomes of *Sorghum halepense* (L.) Pers is an effective fount of antioxidant and anti-diabetic compounds. Further analysis is needed to sharpen its pharmacological activities.

Keywords: *Sorghum halepense* (L.) Pers, Antioxidant, Cytotoxic and anti-diabetic

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Introduction

The Sorghum genus, belonging to the Poaceae, constitute about 44% of weed species that emerge, overrun and cause severe damages to agriculture and livestock in the world [1]. *Sorghum halepense* (L.) Pers, one of its species is known as the most popular weed in the world as it has elevated infestation potential on pastures and crops. Both seeds and rhizomes can grow to reproduce this perennial rhizomatous weed of summer [2]. The habitats of *Sorghum halepense* (L.) Pers includes fields, open forests, wetlands and ditches [3].

It is a rich source of several significant compounds including oxazolidinone, taxiphyllin, chlorogenic acid, sorgoleone prunasin, and dihydrosorgoleone *p*-coumaric acid, hydroxyl benzyl alcohol, *p*-hydroxybenzoic acid, *p*-hydroxybenzaldehyde, *p*- phloroglucinol, aliphatic acids from the rhizomes of *S. halepense (L.) Pers* [4–6].

Plants possess numerous therapeutically active chemical constituents. Most of these compounds produced by the plants are extracted in various solvents and their curative effects are identified.

A chronic metabolic disorder, diabetes mellitus, is characterized by improper regulation of carbohydrate, protein, and lipid metabolism due to malfunction or deficiency in insulin production [7]. It is also associated with hyperglycemia, which in turn, through the non-enzymatic glycation and self-oxidation of glucose [8] leads to oxidative stress [9]. This oxidative stress has been observed to be closely associated with complications of diabetes mellitus, including nephropathy, anemia, hepatopathy [9] dyslipidemia, the primary threat for cardiovascular ailment [10].

Materials and methods

Chemicals and reagents

Analytical grade chemicals including sodium phosphate dibasic, sodium phosphate monobasic, ABTS, DPPH, hydrogen peroxide, *n*-hexane, chloroform, Folin-Ciocalteu reagent, Na_2CO_3, sea salts, gallic acid, SDS, TBA, NaCl, KCl, alloxan, normal saline, ascorbic acid, starch, glucophage, amylase, DNS (dinitrosalicylic) acid, potassium persulfate, Benedict's solution, Ferric chloride, Molish's reagent, sulphuric acid, Libermann- Burchard reagent, Mayer's reagent, were used during biological activities. All the mentioned chemicals were purchased from Sigma-Aldrich except glucophage which was purchased from Merck.

Plant materials

In March 2017, the plant of *Sorghum halepense* (L.) Pers was collected from District Bannu, Pakistan. It was recognized by Prof. Abdur Rehman, Government Post Graduate College Bannu, KPK, Pakistan and deposited a specimen voucher (AR-125) in the herbarium of University of Science and Technology Bannu, KPK, Pakistan. Thereafter their rhizomes were collected and shade dried.

Preparation of extract

The rhizomes of juvenile *Sorghum halepense* (L.) Pers were collected, washed thoroughly, shade dried and pulverized into small pieces by using pestle and mortar. A 70% methanol solution (2 L) was used to extract the active compounds from the pulverized rhizomes (800 g) by submerging it in the mentioned solution with regular shakeup for 72 h at room temperature. It was filtered (Whatman No. 3 filter paper, Whatman Ltd., England), the filtrate was dried under rotary vacuum evaporator (Strike202, Italy) at 40 °C to obtained concentrated sticky methanolic extract (23.56 g) and stored for the future use.

Fraction's preparation

20 g of methanolic extract was sequentially fractionated with *n*-hexane, chloroform and aqueous (400 ml each) by using separating funnel. Filtrates of *n*-hexane (1.87 g), chloroform (4.93 g) and aqueous (7.973 g) fractions were evaporated at room temperature and stockpiled the gummy portion for further analysis.

Phytochemical screening

Methanolic extract of *Sorghum halepense* (L.) Pers rhizomes and its various fractions were phytochemically evaluated to verify the existence or lack of terpenes, cardiac glycosides, flavonoids, steroids, carbohydrate, proteins, saponins and alkaloids according to standard methods [11].

Brine shrimp lethality assay

Methanolic extract and its different fractions of rhizomes of young *Sorghum halepense* (L.) Pers were introduced into the biological analysis of shrimp (*Artemia salina*) mortality to determine their cytotoxic properties [12]. Artificially prepared 4% seawater was poured into a container comprising on two chambers, one covered with aluminum foil and another illuminated with energy saver lamp, suspended I mg brine shrimp egg in a covered chamber for 24 h. The shrimps after hatching crossed the central porous wall and came to the luminous chamber. Different concentrations i.e. 100, 250, 500 and 1000 μg/ ml of each sample were prepared in methanol. The samples (1 ml) were put into the experimental test tubes containing artificial seawater and allowed complete evaporation of methanol from it. The control lacked the samples. Ten shrimps were added to all test tubes, incubated for 24 h and thereafter in each test tube, the live shrimps were counted. The % lethality of shrimps was calculated by Abbot's formula.

$$\%Death = (Sample - control/control) \times 100$$

Total phenolic content

Folin-Ciocalteu reagent [13] was applied for the determination of total phenolic content in the rhizomes of *Sorghum halepense* (L.) Pers methanolic extract and its fractions. The working solution of Folin–Ciocalteu reagent was prepared by its 10X dilution in distilled water whereas 1–5 mg/ml sample solutions were prepared in respective solvents. Sample solution (250 μl) was mixed with a working solution of Folin–Ciocalteu reagents (2.5 ml) and incubated for 5 min at room temperature. 2.5 ml saturated solution (60 mg/ml) of Na_2CO_3 was poured into the reaction mixture and incubated once more at the said temperature for 2 h. Instead of a sample solution, gallic acid was added to standard/ control. The absorbance was measured at 725 nm spectrophotometrically and the findings were shown as gallic acid equivalent.

Antioxidant assays

DPPH assay

A commonly adopted antioxidant assay, DPPH assay was followed according to the standard [14] procedure to find out the antioxidant potential of rhizomes of *Sorghum halepense* (L.) Pers. Different working solutions (125, 250, 500, 1000, 1500 and 2000 μg/ml) of all samples and ascorbic acid were prepared. DPPH solution (3 mg in 100 ml) was prepared, incubated at 25 °C in a baker fully covered with aluminum foil for half an hour; thereafter its absorbance was measured at 517 nm and adjusted it to less than one. The sample /ascorbic acid solutions were poured to DPPH solution and measured the absorbance spectrophotometrically. Their free radical scavenging capabilities were determined as follows.

$$\text{Percent scavenging of DPPH free radicals} = (A_1 - A_2/A_1) \times 100$$

Where A_1 = the control absorbance (DPPH only) and A_2 = the experimental absorbance (DPPH + sample).

ABTS assay

The capability of prepared samples to scavenge ABTS free radical was assessed by using ABTS assay according to the standard procedure [15]. Potassium persulfate solution (2.45 mM) was added to ABTS solution (7 mM) and incubated overnight in the dark and thereafter relevant solvent (1:1) was added to it to manage its absorbance to 0.900 (±0.02) at 745 nm. The sample solution (300 μl: 125–2000 μg/ml in related solvent) was poured into the working solution of ABTS, incubated for 6 min and measured the absorbance. Ascorbic acid was used as a substitute for the sample in a control. The percentage competence of samples/ ascorbic acid to forage ABTS free radicals was computed as follows;

$$\text{Percent scavenging} = [(\text{control absorbance (ABTS)} - \text{sample absorbance}) / (\text{control absorbance})] \times 100$$

H_2O_2 assay

The H_2O_2 scavenging potential of prepared samples was investigated by following the procedure of Wettasinghe and Shahidi; 2000 [16]. 43 mM H_2O_2 solution was prepared in 100 mM phosphate buffer (pH 7.4) while the sample solutions (125–2000 μg/ml) were prepared in relevant solvents. The sample solution was poured into the reaction mixture, incubated for 40 min at room temperature and then measured its absorbance at 230 nm. H_2O was added as a substitute for H_2O_2 in the blank. The percentage effectiveness of samples to scavenge H_2O_2 was computed as follows.

$$\text{Percent scavenging effect} = [(\text{control absorbance } (H_2O_2) - \text{sample absorbance}) /(\text{control absorbance})] \times 100$$

All tests were carried out three times and articulated the findings as means ± SD.

Alpha-amylase inhibition

The capacity of samples to inhibit alpha-amylase was explored according to Worthington Enzyme Manual [17] guideline. Sodium phosphate buffer (pH 6.9, 20 mM with 6 mM NaCl) was used to prepare the dinitrosalicylic (DNS) acid, alpha-amylase and starch solutions. The sample (300 μL), 500 μL starch solution (1%) and 500 μL alpha-amylase solutions (0.5 mg/mL) were put together and kept for 10 min at 25 °C. DNS acid (1.0 mL) was poured into the reaction mixture to stop the enzymatic reaction, incubated in the boiling water for 5 min and subsequently cooled to room temperature. Distilled water (3 ml) was added to all test tubes to dilute the reaction mixture. The absorbance was measured spectrophotometrically at 540 nm. The percentage inhibition of alpha-amylase caused by the samples was calculated as follows.

$$\text{Amylase inhibition (\%)} = [(\text{control absorbance(Blank)} - \text{sample absorbance})/ (\text{control absorbance})] \times 100$$

In vivo study

Experimental animals

The experimental animals, male Wistar Albino rats (260–280 g b.w.) for the current study were bought from animal house, National Institute of Health Sciences (NIH) Islamabad. These animals were kept in cages under a standard husbandry environment (25 ± 0.5 °C; 12 h light/dark cycle), in reference to the guidelines approved by Institutional Animals Ethics Committee

(IAEC number 123) and served with locally (Bannu) purchased pellet diet and fresh tap water. Prior to begin an experiment, the animals were acclimatized to the laboratory conditions in Bannu for 1 week.

Toxicity study

Two testings and one standard group, each with 6 healthy male Wistar Albino rats were fasted during the night and gave free access to the water. Different doses (1 and 2 g/kg b.w.) of methanolic extract of rhizomes of *Sorghum halepense* (L.) Pers were orally administered once (in 1 mL) to experimental groups to assess its possible toxicity. Mortality of the experimental animals as well as modification in their behaviors were examined for 24 h [18] and did not observe mortality or any other symptom of acute toxicity which indicated that LD_{50} of the tested extract is > 2000 mg/kg. Based on the result of an acute toxicity test, different doses (150 and 300 mg/kg) of extract were selected for the assessment of its antidiabetic characteristics.

Induction of experimental diabetes in rats

After fasting for 16 h, a single intra-peritoneal dose of freshly prepared alloxan (120 mg/kg b.w.) in a volume of 1 mL kg^{-1} in normal saline was injected into each experimental rat so as to induce experimental diabetes in them [19–22]. The fasting blood glucose levels of rates were checked after 72 h [23] and rats with fasting blood glucose concentration ≥ 200 mg/dL were considered diabetic (in rats common range of blood glucose is 80–120 mg/dl) [21, 23, 24] and selected for designed experiments.

Experimental procedure

Following the confirmation of induction of diabetes in rats, they were grouped randomly into 5 groups of 5 rats each. All the rats within a group were numbered/marked at their tails with the help of a permanent marker. Group 1: Normal rats (control); Group 2: Untreated diabetic rats (negative control); Group 3: Diabetic rats treated with glibenclamide at 10 mg/kg b.w; Group 4: Diabetic rats treated with methanolic extract of rhizomes of *Sorghum halepense* (L.) Pers at 150 mg/kg b.w; Group 5: Diabetic rats treated with methanolic extract at 300 mg/kg b.w. Plant extract and glibenclamide were fed orally to the rats for 21 days every morning with help of 16 gauge gastric intubation as the people conventionally exercise ethno medicines orally for the treatment of various ailments [25].

Quantification of body weight and blood glucose level

The fasting blood glucose concentrations were examined at the start prior to begin dose supply (t = 0; 1st day) and on 7th, 14th and 21st day of treatment with help of glucometer (Medisign, England). Blood samples from rats were obtained by aseptic puncture of their tail veins. The animals were weighed and articulated their initial (t = 1st day) and final (t = 21st day) weights [26].

Sacrificing animals and serum collection

Following the treatment with extract for 21st day, the rats were fasted overnight and anaesthetized on 22nd day. During anesthesia, each rat was placed in transparent pot which contained small cotton pad soaked with 4 ml diethyl ether (anesthetic ether) in which the rat became anaesthetized and insensible owing to inhalation of mentioned anesthetic drug and subsequently sacrificed. For obtaining serum, the collected blood by puncturing heart was kept in plain sample bottles, allowed to clot for 2 h and thereafter centrifuged for 10 min at 3000 rpm. The serum (supernatant) was collected for advanced investigations.

Lipid profile determination of serum

The level of triglycerides (TG), HDL and total cholesterol in blood serum was estimated with the help of chemistry analyzer (Selectra, XL, Netherlands) using commercially accessible kits (Gesan productions, Italy) in accordance with the manufacturer's protocols. The level of very low density lipoproteins (VLDL) and low density lipoproteins (LDL) was computed by using Friedwald equation.

Assessment of liver and kidneys function

Aspartate and alanine aminotransferase activities (AST and ALT), creatinine and total bilirubin were found with help of chemistry analyzer (Selectra, XL, Netherlands) using commercially existing kits (Gesan productions, Italy) according to the manufacturer's protocols.

Determination of malondialdehyde (MDA)

According to the published protocol, TBARS assay was conducted for the analysis of lipid per oxidation [27, 28]. Ice cold KCl solution (1.5%) was used to prepare 10% homogenate of liver tissues, centrifuged for 10 min at 4000 x g and collected the supernatant. 100 μL of supernatant was put without extract (control) or with extract (experimental) to the test tubes and kept at 37 °C for 60 min. Afterward incubation, 8.1% SDS, acetate buffer and TBA solution were put together and incubated once more for 60 min at 100 °C. The development of light pink color revealed the reaction of TBA with MDA. The absorbance was measured spectrophotometrically at 532 nm after ice cooling the tubes.

Estimation of glutathione level

Moron et al. (1979) protocol of spectrophotometric assay was opted to calculate glutathione (GSH) concentration in tissue homogenate [29]. The reaction of

glutathione (GSH) (acid soluble sulphydryl groups) with DTNB (5, 50-dithiobis-2-nitrobenzoic acid) resulted in yellow color complex and showed the existence of glutathione [29]. During this course, tissue homogenate and 25% TCA were put together in proportion of 5:1 to precipitate the tissue homogenate and followed by centrifugation for 10 min at 3000 rpm. Subsequently, the 100 μL (supernatant) was mixed with the reaction mixture containing on 2 mL (0.6 mM) DTNB and 0.9 mL of sodium phosphate buffer (0.2 mM, pH 7.4) whereas the blank lack the supernatant. The development of yellow color complexes were measured against the blank at 412 nm. The molar extinction coefficient of DTNB (13,100/ Mcm) was used for the estimation of glutathione level.

Determination of superoxide dismutase (SOD)

Beauchamp and Fridovich (1971) method was used to evaluate the activity of superoxide dismutase [30]. During the current experiment, the reaction mixture was comprised on 50 mM sodium carbonate (1 mL), 0.1 mM EDTA (200 μL), tissue homogenate (500 μL) and 400 μL of 25 μM NBT (nitro blue tetrazolium). The reaction was initiated by mixing 1 mM (400 μL) hydroxylamine–hydrochloride with the reaction mixture and measured the change in absorbance at 560 nm spectrophotometrically. The activity of SOD was measured as its quantity in units / ml that restrains 50% NBT reduction. The results of test (receiving extract) and normal groups were compared. The improved action of SOD shows an antioxidant characteristic.

Catalase activity

The serum catalase activity was determined by following the reported procedure of Atawodi [31]. 10 μL serum was mixed with 2.80 mL potassium phosphate buffer (50 mM, pH 7.0) followed by addition of 0.1 mL of fresh 30 mM H_2O_2 to commence the reaction. The breakdown rate of H_2O_2 was measured spectrophotometrically for 5 min at 240 nm and calculated the activity of catalase by applying the molar extinction coefficient of 0.041 mM / cm where its amplified activity indicates an antioxidant effect.

Statistical analysis

GraphPad prism software was used to analyze the results. All in vitro experiments were performed in triplicate while quintuple in vivo and showed the results as mean ± standard deviation. Furthermore, Pearson correlation coefficient was calculated between total phenolic versus antioxidant and antidiabetic activities. One way ANOVA and Dunnett's-test were used for in vivo analysis. The $p < 0.05$ was considered statistically significant.

Results

Phytochemical assessment

The phytochemical assessment of methanolic extract of rhizomes of *Sorghum halepense* (L.) Pers and its different fractions uncovered the presence of cardiac glycosides, flavonoids, terpenes, steroids, carbohydrates, proteins and alkaloids in methanolic extract. Carbohydrates and steroids were only present in aqueous and chloroform fractions respectively while both fractions contained proteins and alkaloids. Cardiac glycosides were absent in the aqueous fraction while flavonoids in the chloroform fraction (Table 1).

Brine shrimp lethality assay

The brine shrimps lethality bioassay revealed the cytotoxic characteristics of methanolic extracts of rhizome *Sorghum halepense* (L.) Pers and its different fractions. 70.5 ± 1.2, 50.4 ± 1.1, 50.3 ± 1.1 and 40.3 ± 1.6% lethality of brine shrimp against methanolic extract and its aqueous, *n*-hexane and chloroform fractions at the level of 1000 μg/ml was found respectively (Table 2).

Total phenolic contents

The rhizome's extract of *Sorghum halepense* (L.) Pers was explored for total phenolic content by using Folin Ciocalteu phenol reagent and gallic acid (standard). The contents of phenolic compounds in therapeutic plants are vastly capricious i.e. 2.34–152.32 mg GAE/g [32]. In the present project, the highest amount of phenolic contents was present in methanolic extract (28.30 ± 1.3) while lowest in *n*-hexane fraction (8.87 ± 1.35 mg GAE/g). Table 3 shows the results.

Antioxidant assays

Plants are the major sources of natural antioxidants. The said antioxidants scavenge free radicals and their reactive derivatives (ROS), which are accountable for different disorders in human beings [33]. The mentioned ability of medicinal compounds was estimated by using the commonly used standard assays.

DPPH method

The estimation of primary antioxidant characteristics of plant extracts was found by opting DPPH assay where free radicals of DPPH are reduced. The antioxidant activities of methanolic extract of rhizomes of *Sorghum halepense* (L.) Pers, its prepared fractions and ascorbic acid (standard) were measured spectrophotometrically at 517 nm and compared [34]. Antioxidant capabilities of standard and samples are shown in Fig. 1.

The highest antioxidant activity was shown by methanolic extract (40.02%) followed by chloroform fraction (33.11%), aqueous fraction (32.86%), *n*-hexane fraction (23.79%) at the concentrations of 2 mg/ml. The ascorbic

Table 1 Phytochemical evaluation of rhizomes of *Sorghum halepense* (L.) Pers methanolic extract and its aqueous, chloroform and *n*-hexane fractions

S#	Phytochemical	Methanolic extract and its fractions			
		Methanolic extract	Aqueous fraction	Chloroform fraction	*n*-Hexane fraction
1	Cardiac glycosides	+	−	+	+
2	Flavonoids	+	+	−	+
3	Terpenes	+	−	−	+
4	Steroids	+	−	+	−
5	Saponins	−	−	−	−
6	Carbohydrate	+	+	−	−
7	Proteins	+	+	+	−
8	Gums	−	−	−	−
9	Alkaloids	+	+	+	−

Performed the experiments in triplicate ($n = 3$)

acid (standard) indicated 53.43% antioxidant activity at the same concentration. The antioxidant properties of methanolic extract of rhizome of *Sorghum halepense* (L.) Pers and its fractions were found concentration dependant.

ABTS radical cation assay

It is an easy and suitable assay for both hydrophilic and lipophilic antioxidants. During this assay, ABTS and potassium persulfate react together and a blue chromophore (ABTS•+) is generated. Plant extract or ascorbic acid (standard antioxidant) reduces the ABTS• + cation radical [35]. During the current study, methanolic extract (40.48%) exhibited the highest antioxidant activity followed by chloroform fraction (35.41%), an aqueous fraction (33.18%) and *n*-hexane fraction (23.81%) at the level of 2 mg/ml. The mentioned prospective of ascorbic acid (57.28%) was also articulated (Fig. 2).

Hydrogen peroxide (H_2O_2) scavenging capacity

The prepared plant extract of rhizome of *Sorghum halepense* (L.) Pers indicated scavenging of H_2O_2 in a concentration-dependent manner. The methanolic extract (50.85%), aqueous fraction (42.90%), *n*-hexane fraction (17.58%) and ascorbic acid (standard) expressed (52.22%) scavenging activity respectively at the concentration of 2 mg/ml. The chloroform fraction did not exhibit H_2O_2 scavenging potential at the mentioned concentration (Fig. 3).

Alpha-amylase inhibition

The results of alpha-amylase inhibition by methanolic extract of *Sorghum halepense* (L.) Pers rhizome and its different fractions were indicated in Fig. 4. The mentioned properties of available commercial medicine, glucophage (standard), the methanolic extract and chloroform fraction were measured 63.14%, 61.87%) and 22.66% respectively.

Correlation of total phenolic contents to antioxidant and antidiabetic potency

The quantified total phenolic contents were correlated with percent antioxidant activities of extracted samples and found significant. The non-significant correlation was found between total phenolic contents and antidiabetic potential of samples used (Pearson correlation, two-tailed). Results were articulated in Table 4.

Table 2 Percentage fatality of brine shrimps expressed by methanolic extract and its different fraction of rhizome of *Sorghum halepense* (L.) Pers

Concentration (μg/ml)	% age lethality by methanolic extract and its fractions				Control
	Methanolic extract	Chloroform fraction	Aqueous fraction	n-hexane fraction	
100	40.4 ± 1.3 ^{a}	30.3 ± 1.3 ^{c}	20.5 ± 1.6	10.3 ± 1.5	00 ± 00
250	50.6 ± 1.1 ^{a}	30.5 ± 1.2 ^{c}	20.4 ± 1.2	10.4 ± 1.3	00 ± 00
500	50.3 ± 1.4 ^{a}	40.6 ± 1.4 ^{c}	40.2 ± 1.4	30.5 ± 1.7	00 ± 00
1000	70.5 ± 1.2 ^{a}	50.4 ± 1.1 ^{c}	50.3 ± 1.1	40.3 ± 1.6	10.2 ± 1.1

All the treated groups were compared with the control group. c indicates significance at $P < 0.05$ and a at $p < 0.001$ (Dunnett's-test). Performed the experiments in triplicate ($n = 3$)

Table 3 Total phenolic content (mg GAE/g) of methanolic extract and its different fractions

Extracts	Methanolic extract	Chloroform fraction	Aqueous fraction	*n*-Hexane fraction
Total phenolic content	28.30 ± 1.3	17.34 ± 1.43 [c]	12.7 ± 1.32	8.87 ± 1.35 [a]

The total phenolic content in methanolic extract was compared with other fractions. c indicates significance at $P < 0.05$ and a at $p < 0.001$ (Dunnett's-test). Performed the experiments in triplicate ($n = 3$)

In vivo study

Test for acute toxicity

A single oral dose of *Sorghum halepense* (L.) Pers methanol extract (2 g/kg) showed no mortality or acute toxicity symptoms in rats over 24 h. Acute toxicity appraisal has shown that *Sorghum halepense* (L.) Pers rhizome extract is safe up to 2 g/kg b.w. and the approximate LD_{50} is more than 2 g/kg.

Effect of a methanol extract of Sorghum halepense (L.) Pers rhizomes on body weight of diabetic rats

The current evaluation indicated a significant ($P < 0.05$) decline in the weight of diabetic control group with respect to the normal control group on 1st and 21st days of treatment. Treatment of diabetic rats with a methanol extract of *Sorghum halepense* (L.) Pers rhizomes, reliably ($P < 0.05$) recovered their body weights with regard to diabetic controls (Fig. 5). The extract at dosage of 300 mg/kg and glibenclamide (10 mg/kg) showed a marked increase in body weight of rats with diabetes on 21st day (Fig. 5).

Effect of methanol extract of Sorghum halepense (L.) Pers rhizomes on blood glucose

Treatment of diabetic rats with *Sorghum halepense* (L.) Pers rhizome extract expressed significant ($p < 0.001$) reductions in their blood glucose levels as shown in Fig. 6; thereby expressing the applied extract as a hypoglycemic agent. The diabetic control group demonstrated imperative increases in the concentration of blood glucose during the experiment as compared to the normal control group. Conversely, the rats nourished with a higher dose (300 mg/kg) of extract showed an obvious reduction in the concentration of blood glucose, followed lower dose (150 mg/kg) and standard group (10 mg/kg) as shown in Fig. 6.

Effect of methanol extract of Sorghum halepense (L.) Pers rhizomes on rat's liver function

In experimental animals, alloxan attacks the pancreas and also causes damage to other organs such as the kidneys and liver. Serum levels of biochemical markers; AST, ALT, total bilirubin and ALP, which are extremely sensitive to oxidative stress and poisonous chemicals were used to assess liver damage. Protective effects of methanol extract of rhizomes of *Sorghum halepense* (L.) Pers and glibenclamide against changes in liver serum markers are shown in Table 5. Treatment of rats with alloxan considerably raised the activity of marker enzymes in liver serum,

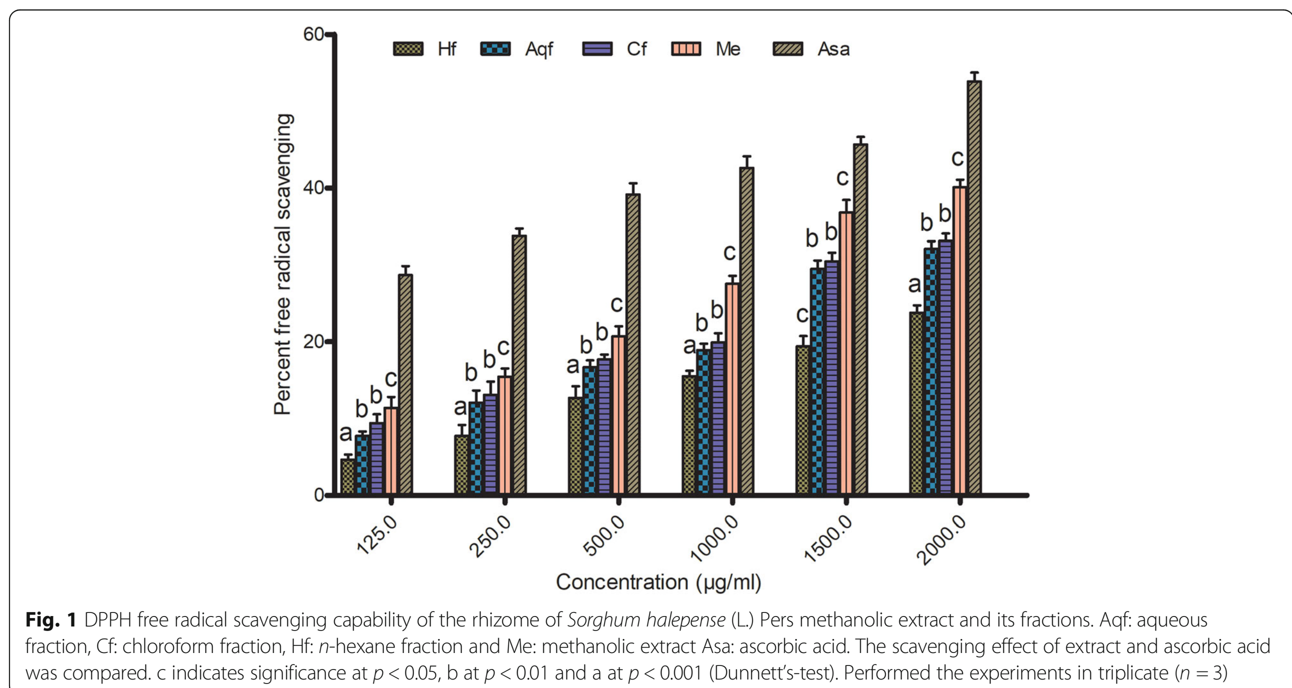

Fig. 1 DPPH free radical scavenging capability of the rhizome of *Sorghum halepense* (L.) Pers methanolic extract and its fractions. Aqf: aqueous fraction, Cf: chloroform fraction, Hf: *n*-hexane fraction and Me: methanolic extract Asa: ascorbic acid. The scavenging effect of extract and ascorbic acid was compared. c indicates significance at $p < 0.05$, b at $p < 0.01$ and a at $p < 0.001$ (Dunnett's-test). Performed the experiments in triplicate ($n = 3$)

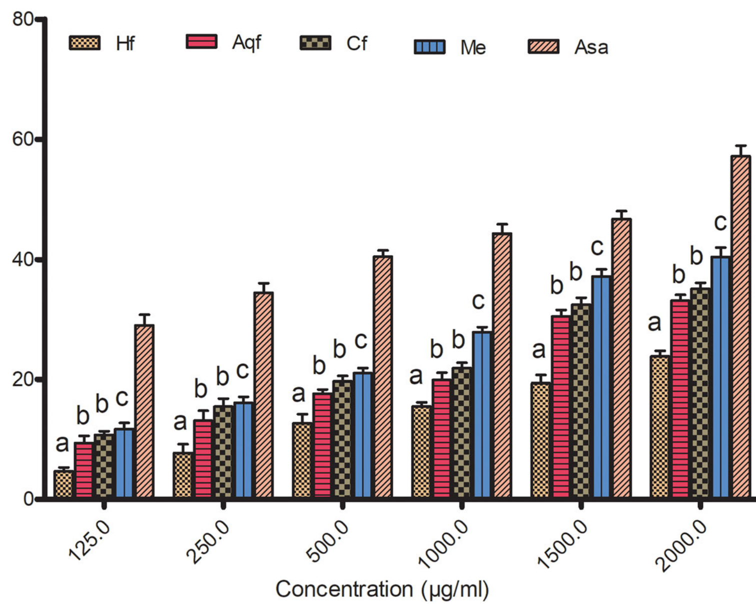

Fig. 2 ABTS free radical scavenging capability of the rhizome of *Sorghum halepense* (L.) Pers methanolic extract and its fractions. Aqf: aqueous fraction, Cf: chloroform fraction, Hf: *n*-hexane fraction and Me: methanolic extract Asa: ascorbic acid. The scavenging effect of extract and ascorbic acid were compared. c indicates significance at $p < 0.05$, b at $p < 0.01$ and a at $p < 0.001$ (Dunnett's-test). Performed the experiments in triplicate ($n = 3$)

which was significantly recovered ($P < 0.05$) as compared with the control group after oral administration of *Sorghum halepense* (L.) Pers rhizome extract. Similarly, the intoxication of alloxan was recovered significantly ($p < 0.05$) by treatment with glibenclamide (10 mg/kg b.w.).

Effect of methanolic extract of Sorghum halepense (L.) Pers rhizomes on triglycerides, LDL cholesterol, total cholesterol, HDL cholesterol and serum VLDL cholesterol

Free radicals cause lipid per-oxidation and affect the lipid profile especially when hepatotoxin react with

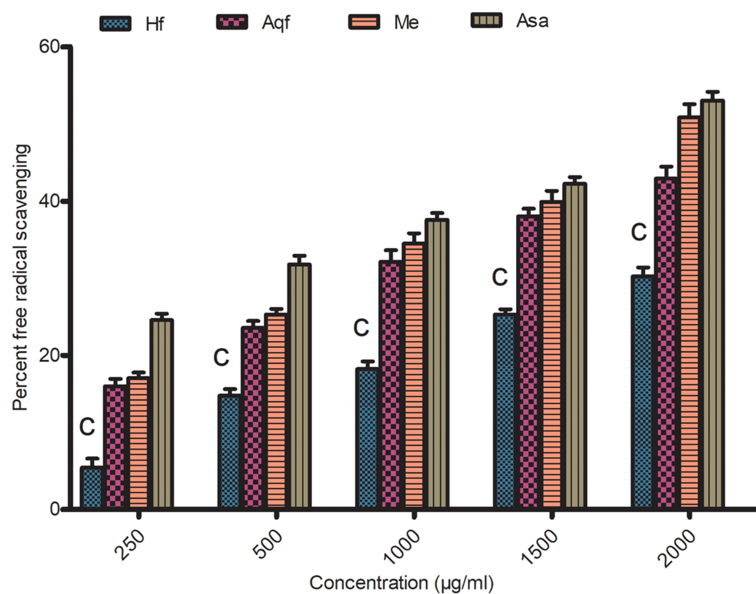

Fig. 3 H_2O_2 free radical scavenging capability of the rhizome of *Sorghum halepense* (L.) Pers methanolic extract and its fractions. Aqf: aqueous fraction, Cf: chloroform fraction, Hf: *n*-hexane fraction and Me: methanolic extract Asa: ascorbic acid. The scavenging effect of extract and ascorbic acid were compared. c indicates significance at $p < 0.05$ (Dunnett's-test). Performed the experiments in triplicate ($n = 3$)

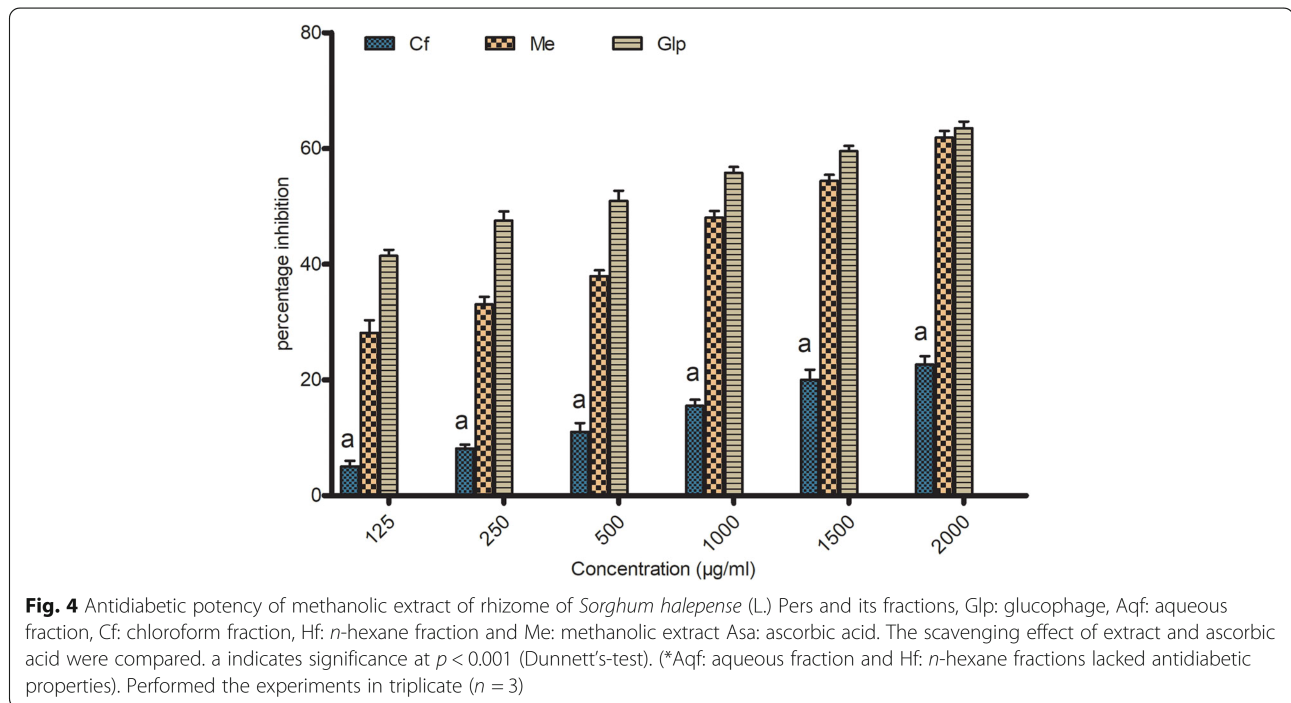

Fig. 4 Antidiabetic potency of methanolic extract of rhizome of *Sorghum halepense* (L.) Pers and its fractions, Glp: glucophage, Aqf: aqueous fraction, Cf: chloroform fraction, Hf: *n*-hexane fraction and Me: methanolic extract Asa: ascorbic acid. The scavenging effect of extract and ascorbic acid were compared. a indicates significance at $p < 0.001$ (Dunnett's-test). (*Aqf: aqueous fraction and Hf: *n*-hexane fractions lacked antidiabetic properties). Performed the experiments in triplicate ($n = 3$)

polyunsaturated fatty acids. In the contemporary evaluation, the levels of serum VLDL cholesterol, triglycerides, HDL cholesterol, total cholesterol and LDL cholesterol are summarized in Table 6. Elevated levels of lipid parameters such as triglyceride, total cholesterol, LDL cholesterol and VLDL cholesterol were present in the serum of diabetic rats, while HDL cholesterol level's declined. These parameters were reliably recovered depending on the dose in groups treated with the extract (150 and 300 mg/kg of b.w.) of the rhizomes of *Sorghum halepense* (L.) Pers.

Effect of a methanol extract of Sorghum halepense (L.) Pers rhizomes on urea and creatinine in rat serum

Changes in serum creatinine, total protein and urea concentration in normal, diabetic and treated groups are illustrated in Table 7. Protective effect of rhizomes of *Sorghum halepense* (L.) Pers extract at concentrations of 150 and 300 mg/kg b.w. was observed where the increased serum level of the renal profile was reliably ($p < 0.05$) restored; serum creatinine, urea, and a decrease in total serum protein in treatment groups.

Table 4 The correlation between total phenolic content quantified in methanolic extract of rhizome of *Sorghum halepense* (L.) Pers and its different soluble fractions and their antioxidant and antidiabetic potency. *P*-value (two-tailed) and ns: none significant

	Assays	Correlation R^2 Phenolics	Significance
1	% DPPH radical scavenging potential	0.8081	yes
2	% ABTS scavenging capability	0.8147	yes
3	% H_2O_2 scavenging	0.8023	yes
4	% amylase inhibition	0.6383	No

Antioxidant activities of a methanol extract of Sorghum halepense (L.) Pers rhizomes in vivo

The present study showed that a methanol extract of *Sorghum halepense* (L.) Pers rhizomes effectively neutralized alloxan-induced oxidative stress in diabetic rats.

In the normal control (non-diabetic rats), the level of MDA was very low than diabetic control (untreated diabetic rats). The standard, glibenclamide, fairly lessen the level of MDA, while a higher dose (300 mg/kg b.w.) of the plant extract appreciably ($P < 0.05$) reinstated to an almost normal state. The concentration/level of MDA and glutathione has indirect proportionality; diabetic control (diabetic rats) indicated the highest level of MDA (2.95 ± 0.35 nmol / ml) and the lowest level of glutathione (23.2 ± 3.19 µmol/mg protein) as compared to the normal group. Treatment with the applied extract recovered the glutathione concentration in diabetic rats, since the extract has antioxidant characteristics.

The concentration of superoxide dismutase (SOD) and catalase, antioxidant enzymes, in the liver tissue homogenate decreased to 27.8 ± 4.15 and 21.2 ± 2.16 respectively when compared the diabetic control group with the normal group (SOD: 47.2 ± 4.38, catalase: 36 ± 2.23). The extract treatment caused a significant ($P < 0.05$) recovery in catalase and SOD levels. The resulting effects of

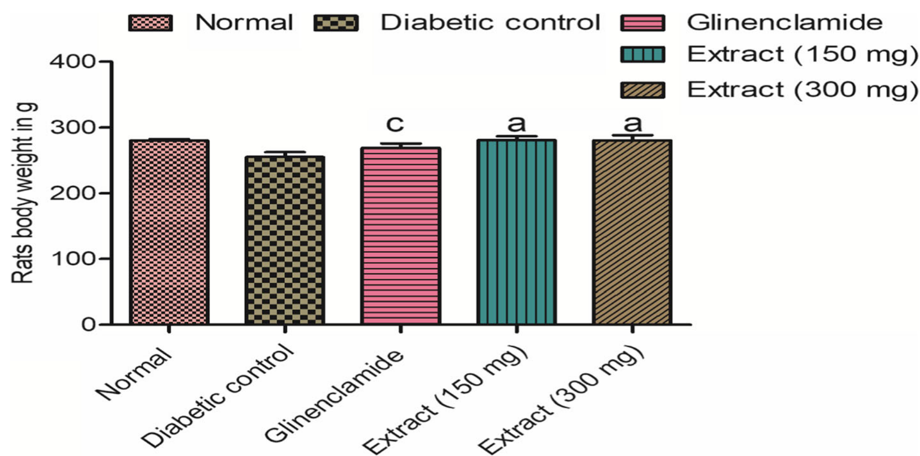

Fig. 5 Represent the effect of methanolic extract of rhizomes of *Sorghum halepense* (L.) Pers on body weight of diabetic rats. Represented the data ($n = 5$) as Mean ± SD, a shows significance at $P < 0.001$ and c at $P < 0.05$ (Dunnett's-test), the normal group was compared with the untreated group and in turn its comparison was made with extract treated group and standard

Sorghum halepense (L.) Pers rhizome extract affects the comparative levels of glutathione, MDA, SOD and catalase (Table 8).

Discussion

Phytochemical components in the plant extracts are considered to be active biologically and are accountable for various actions like antidiabetic, anticancer, antifungal, anti-inflammatory and antibacterial [36, 37]. Two types of metabolites are produced by a plant, primary metabolites i.e. lipids, carbohydrates and proteins and secondary metabolites which include alkaloids, phenolics, terpenes, essential oils, tannins, flavonoids, sterols. The literature study revealed that natural compounds, secondary metabolites played a significant role in the healing of various disorders [38, 39]. Typically, the extraction of the secondary metabolite is based on the polarity of the solvent and its interaction with preferred compounds [40, 41]. The phytochemical analysis of rhizome's methanolic extract of *Sorghum halepense* (L.) Pers showed the presence of flavonoids, cardiac glycosides, terpenes, carbohydrates, steroids, alkaloids and proteins. The methanolic extract lacked saponins and gums whereas aqueous and chloroform fractions contained carbohydrates and steroids respectively.

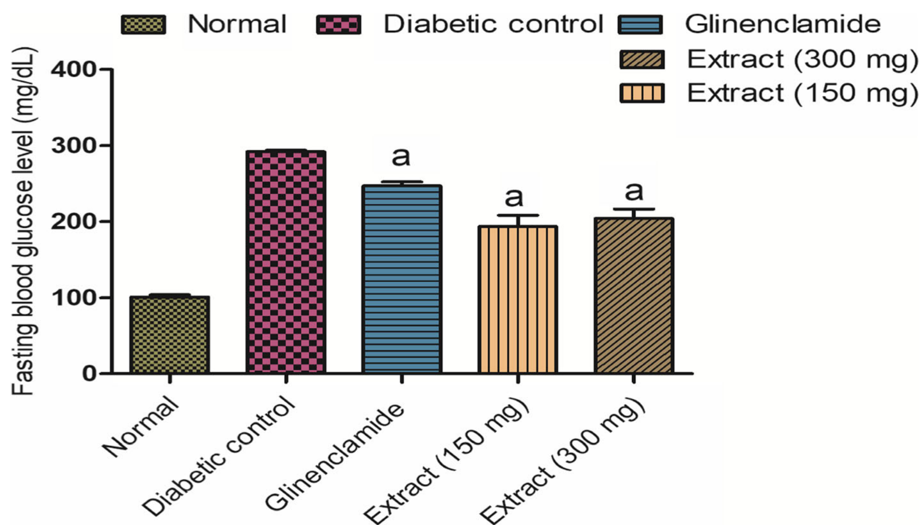

Fig. 6 Effect of *Sorghum halepense* (L.) Pers extract on blood glucose levels of diabetic rat; Represented the data ($n = 5$) as Mean ± SD, a shows significance at $P < 0.001$ (Dunnett's-test), the normal group was compared with untreated group and in turn, its comparison was made with extract treated group and standard

Table 5 The potency of methanolic extract of rhizomes of *Sorghum halepense* (L.) Pers to normalize the level of ALT, ALP, AST and total bilirubin in serum

	ALT (μmol/L)	ALP (μmol/L)	Total bilirubin (μmol/L)	AST (μmol/L)
Normal control	145 ± 1.9	217 ± 2.86	1.09 ± 0.27	65.0 ± 3.16
Diabetic control	236 ± 3.4	416 ± 3.7	1.96 ± 0.38	107.0 ± 4.62
Glibenclamide (10 mg)	158 ± 2.9 [a]	298 ± 5.72 [a]	1.22 ± 0.08 [a]	96.4 ± 2.41 [a]
Extract (150 mg)	153 ± 3.8 [a]	285 ± 4.93 [a]	1.14 ± 0.21 [a]	86.8 ± 3.35 [a]
Extract (300 mg)	147 ± 3.6 [a]	224 ± 4.45 [a]	1.00 ± 0.16 [a]	68.4 ± 3.36 [a]

Represented the data ($n = 5$) as Mean ± SD, a shows significance at $P < 0.001$ (Dunnett's-test), the normal group was compared with untreated group and in turn its comparison was made with extract treated group and standard

On other hand, the cardiac glycosides were missing in the aqueous fraction whilst flavonoids in the chloroform fraction (Table 1). Comparable results were reported in earlier studies of *Salix mucronata* and *Datura metel L* [42, 43]. A number of reports on phenolic compounds, like terpenoids and flavonoids showed their strong biological efficacies like antidiabetic, antioxidant and anticancer [44, 45]. The antibacterial, antimalarial, cytotoxic and anticancerous characteristics of alkaloids [46] and Na-K-ATPase inhibitory potential of cardiac glycosides [47] has been reported. Similarly, common phenolic compounds, flavonoids are commonly found in the plants [48] and have antioxidant, antiallergic, antibacterial, antiviral, antineoplastic, antidiarrheal, antithrombotic, anti-inflammatory and vasodilatory properties [49]. Furthermore, significant antidiabetic characteristics of the plant based bioactive compounds including phenolic compounds, alkaloids, flavonoids, tannins, terpenoids, glycosides have been reported [50–52]. The incidence of the mentioned secondary metabolites in the plant extract and its fractions indicated that *Sorghum halepense* (L.) Pers may have cytotoxic, antioxidant antidiabetic capabilities associated with different diseases as mentioned in preceding studies [53–55]. The cytotoxic capabilities of methanolic extract and its fractions were determined by brine shrimp lethality bioassay. The methanolic extract and its chloroform, aqueous and *n*-hexane fractions caused brine shrimp lethality up to 70.5 ± 1.2%, 50.4 ± 1.1%, 50.3 ± 1.1% and 40.3 ± 1.6% respectively at the amount of 1000 μg/ml (Table 2). It proposes that the plant extract have credible antimicrobial constituents. Consistent results obtained during the studies of *Coscinium blumeanum, Fibraurea tinctoria and Arcangelisia flava* [56] and *Hapllophyllum tuberculatum* [57].

The present results (Table 3) revealed that the methanolic extract has the highest amount of total phenolic content (28.30 ± 1.3 mg GAE/g) followed by chloroform fraction (17.34 ± 1.43 mg GAE/g), an aqueous fraction (12.7 ± 1.32 mg GAE/g) and *n*-hexane fraction (8.87 ± 1.35 mg GAE/g).

Several existing reports confirm that the plant based extracted and isolated phenolic compounds have phenolic hydroxyl groups; can contribute hydrogen atom or unpaired electron and hence neutralize the free radicals efficiently [58, 59]. The present antioxidant compounds in various plants and even different parts of the same plant have different natures and quantities. Therefore, it is essential to adopt more than one assay to authenticate the antioxidant capability of tested samples [60]. The capabilities of methanolic extract of rhizomes of *Sorghum halepense* (L.) Pers and its *n*-hexane, chloroform and aqueous fractions to scavenge free radical was assessed by choosing commonly used standard assay i.e. DPPH, ABTS and H_2O_2 assays [61]. The free radicals produced during these assays were scavenged by the antioxidant constituents in the plant extract. In DPPH assay, DPPH (a, a-diphenyl-b-picrylhydrazyl) is changed

Table 6 Consequences of rhizomes of *Sorghum halepense* (L.) Pers extract on the concentration of serum's LDL cholesterol, triglycerides, VLDL cholesterol, total cholesterol and HDL cholesterol

Groups	TG (mg/dL)	Cholesterol (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)	HDL (mg/dL)
Normal control	96.0 ± 1.9	88 ± 1.9	27.0 ± 2.1	19.0 ± 0.38	45.0 ± 5.8
Diabetic control	175.0 ± 1.1	97 ± 3.2	49.0 ± 1.9	35.0 ± 2.3	28.0 ± 1.7
Glibenclamide (10 mg)	112.0 ± 4.2 [a]	93 ± 2.9 [a]	32.0 ± 2.6 [a]	22.0 ± 0.84 [a]	36.0 ± 2.4 [a]
Extract (150 mg)	111.0 ± 7.02 [a]	96 ± 4.0 [a]	33.0 ± 4.6 [a]	22.12 ± 1.40 [a]	39.2 ± 2.77 [a]
Extract (300 mg)	105.0 ± 6.73 [a]	93 ± 6.2 [a]	29.0 ± 3.2 [a]	21.08 ± 1.35 [a]	43.4 ± 4.5 [a]

Represented the data ($n = 5$) as Mean ± SD, a shows significance at $P < 0.001$ (Dunnett's-test), the normal group was compared with the untreated group and in turn, its comparison was made with extract treated group and standard, *LDL* Low density lipoprotein, *TG* Triglycerides, *VLDL* Very low density lipoprotein, *HDL* High density lipoprotein

Table 7 Consequences of rhizomes of *Sorghum halepense* (L.) Pers extract on serum level of serum creatinine and urea

	Creatinine (mg/dL)	Urea (mg/dL)
Normal control	0.64 ± 0.05	36.6 ± 1.6
Diabetic control	1.56 ± 0.24	53 ± 4.18
Glibenclamide (10 mg)	1.04 ± 0.24 [a]	42.4 ± 2.40 [a]
Extract (150 mg)	0.8 ± 0.1 [a]	39.8 ± 3.27 [a]
Extract (300 mg)	0.7 ± 0.1 [a]	37.4 ± 3.04 [a]

Represented the data ($n = 5$) as Mean ± SD, [a]shows significance at $P < 0.001$ (Dunnett's-test), the normal group was compared with untreated group and in turn its comparison was made with extract treated group and standard

into a, a-diphenyl-b-picrylhydrazine along with alteration in its color indicating the scavenging potential of the plant extract and is measured spectrophotometrically. ABTS assay is suitable for both hydrophilic and lipophilic antioxidants [62]. In this essay, ABTS and potassium persulfate react together to generate a blue chromophore (ABTS•+). The mentioned cation is reduced by reacting with plant extract or standard antioxidant (ascorbic acid) [35]. H_2O_2 by itself is a puny oxidizing agent but through the oxidation of essential thiol (–SH) groups of enzymes, it can inactivate few enzymes directly. It can enter into the cell by crossing the cell membranes easily. Hydrogen peroxide is catalyzed to hydroxyl radicals and singlet oxygen subject to its exposure to transition metal ions. Singlet oxygen is more toxic to the cellular system than H_2O_2 by itself. Also the hydroxyl radical may be the cause of its various poisonous effects [63], hence it is vital for cells to control the quantity of H_2O_2 biologically.

The literature study showed that the aforementioned available compounds in the extract of rhizomes of *Sorghum halepense* (L.) Pers have the ability to donate hydrogen ions and hence de-colorization of DPPH and ABTS solution [11, 53, 55, 64]. The highest free radicals scavenging potential of methanolic extracts were found 40.02%, 40.48% and 50.85% in DPPH, ABTS and H_2O_2 assays (Fig. 1, 2 and 3) respectively. The difference in free radical scavenging might be owing to the variations in the number of aromatic rings, nature of hydroxyl groups and molecular weight as well as with the number of active components in the extract and its fractions which change their concentrations by fractionation [54, 55, 64]. The elevated antioxidant capacity of methanolic extract might be owing to more phenolic contents in the mentioned extract as compared to its *n*-hexane, chloroform and aqueous fractions. The correlation of total phenolic content with the antioxidant activities (Table 4) was found significant. ($R^2 = 0.8081$, 0.81 47 and 0.8023 for DPPH, ABTS and H_2O_2). These correlations predict that antioxidant characteristic is reliant on phenolic content of a sample. Congruent correlations between antioxidant activities and phenolic contents of different types of sorghum were reported previously [65, 66]. Moreover, strong correlations between phenolic content and antioxidant activities in other cereals such as finger millet and wheat have been documented [67, 68].

Today in the world, diabetes is a major degenerative problem resulting in a number of complications like hypertension, atherosclerosis and microcirculatory disorders [69]. The α-amylase catalyzes the hydrolysis of α-(1, 4)-D-glycosidic linkages of starch and oligosaccharides and liberate monosaccharides in the intestine and thus contribute to hyperglycemia in diabetes. It can be limited by restraining α-amylase in the intestine which slows down the decomposition of starch and oligosaccharides to monosaccharides, reduces assimilation of glucose and consequently decrease postprandial blood glucose level [70]. The mechanism of anti-hyperglycemic potential of extract is unknown. Possibly, it might be due to the presence of flavonoids, terpenes, tannins and alkaloids which might have caused alpha-amylase inhibition. On the basis of their strong binding ability with proteins to form an insoluble and indigestible complexes, they are extensively used as inhibitors [71]. Further, it could elucidate that the phenolics compounds are not the only contributor to antioxidant activity but also causes enzyme inhibition. The α-amylase inhibition might depend on different factors like the methoxy groups, hydroxyl position and lactone rings or the interaction between compounds [72].

The antidiabetic properties of available commercial medicine, glucophage (standard), methanolic extract and

Table 8 Consequences of rhizomes of *Sorghum halepense* (L.) Pers extract on SOD, MDA, glutathione and catalase

	MDA (nmol/ml)	SOD (U min/mg of Protein)	Catalase (U min/mg of Protein)	GSH (μmole/mg of protein)
Normal control	1.64 ± 0.39	47.2 ± 4.38	36 ± 2.23	44 ± 2.23
Diabetic control	2.95 ± 0.35	27.8 ± 4.15	21.2 ± 2.16	23.2 ± 3.19
Glibenclamide (10 mg)	2.04 ± 0.21 [a]	36.2 ± 3.03 [a]	27 ± 3.4 [a]	33 ± 3.16 [a]
Extract (150 mg)	2.08 ± 0.17 [a]	35.6 ± 2.91 [a]	28 ± 2.23 [a]	33.4 ± 2.70 [a]
Extract (300 mg)	1.98 ± 0.21 [a]	39.4 ± 2.30 [a]	31 ± 3.16 [a]	39 ± 2.12 [a]

Represented the data ($n = 5$) as Mean ± SD, a shows significance at $P < 0.001$ (Dunnett's-test), the normal group was compared with the untreated group and in turn, its comparison was made with extract treated group and standard, *MDA* Malondialdehyde, *SOD* Superoxide dismutase

chloroform fraction were measured (63.14%), (61.87%) and (22.66%) respectively (Fig. 4). The anti-diabetic capacity of methanolic extract and standard were close and comparable. The percent inhibition of alpha-amylase was found to be dose-dependent. Similar results were also recorded during the in vitro study of *Solaria cuspidate* leaves [73]. It assumes that the said extract is a valuable source of significant antidiabetic components and was subjected to in vivo study.

The key organ, pancreas determines the energy and dietary state of the body via blood glucose level and secret insulin in response to a raise in blood glucose level [69]. In a situation, where a number of functional beta cells become too limited to produce enough insulin to carry out the body necessities, insulin-dependent diabetes results [74]. Alloxan (beta-cytotoxin), due to its deleterious properties to pancreatic β-cells is renowned for inducing experimental diabetes in several animal species including rats. In this study, alloxan was used to induce diabetes in rats [75] and thereafter treated with different dozes of plant extract and standard drug (glibenclamide). On 21st day of treatment, a substantial ($P < 0.01$) decline in the body weight of the diabetic control group was observed with respect to normal control group whereas the groups nourished with the methanolic extract of *Sorghum halepense* (L.) Pers significantly ($P < 0.05$) reinstated their body weights with respect to diabetic control (Fig. 5). The higher dose (300 mg/kg) of extract indicated considerable development in the weight of diabetic rats at the same interval. Furthermore, comparable increase ($P < 0.05$) in body weight of glibenclamide treated group was noticed on comparison with diabetic control group (Fig. 5) at the same interval. The restorative effects of *Sorghum-tigernut Ibyer* extract on changed glucose concentration, tissue and enzyme damages, and loss of weight in diabetic rats was determined by Shiekuma and coworker [76]. The treatment of diabetics rats with methanolic extract of *Sorghum halepense* (L.) Pers (150 and 300 mg/kg b.w; Fig. 6) for 21 days significantly ($P < 0.05$) decreased the increased concentration of glucose in serum. This decline in serum glucose level was determined from the difference between the initial and final fasting concentrations of glucose in serum and was compared with diabetic control and reference standard. Comparable findings were found during evaluation of antidiabetic properties of *Sorghum bicolor* grains [77].

Usually the increase in aminotransferases is a well-known clue of liver dysfunction and is more frequent in diabetic patients than the common population. Moreover, several diabetic complications such as neuropathy, retinopathy and restricted mobility of joints are associated with the function of liver enzymes, regardless of body mass index, alcohol consumption and metabolic control of diabetes [78]. Significant raise in action of several enzymes such as beta-glucuronidase, N-acetyl-beta-glucosaminidase, leucine aminopeptidase and lysosomal acid phosphatase and cathepsin D have been observed following the injection of alloxan in previous studies [79]. Liver dysfunction under diabetic condition leads to increased activities of alkaline phosphatase (ALP), alanine aminotransferase (ALT), total bilirubin and aspartate aminotransferase (AST) with respect to non diabetes (Table 5). In diabetic animals, the alteration of enzymes in serum are directly associated with the metabolic variations wherein these enzymes are concerned. In the lack of insulin, the elevated actions of transaminases are owing to more availability of amino acids in diabetes and are responsible for the increased ketogenesis and gluconeogenesis found in diabetes. During the recent study, the oral feeding of extract (150 and 300 mg/kg b.w.) has restored the concentrations of ALT, total bilirubin, ALP and AST as indicated in Table 5 [80]. Hence, the obvious restoration in the concentration of the mentioned enzymes (Table 5) was the effect of better metabolism of proteins, fats and carbohydrates. Following the treatment, the revival of ALT and bilirubin levels also indicated the recovery of insulin secretion. In alloxan induced diabetic rats, the plant extracts have revealed the restoration of altered levels of ALT, ALP, total bilirubin and AST in earlier studies [81, 82]. Raise in serum urea and creatinine levels owing to diabetic hyperglycemia were considered the significant indicators of renal dysfunction [83, 84] and reveals a decline in the rate of glumerular filtration. The level of creatinine and urea was normalized significantly ($P < 0.05$) by treating the rats with methanolic extract (Table 7). Harmonious consequences were found in the antidiabetic assessment of three varieties (Heuin sorghum, Chal sorghum and Hwanggeumchal sorghum) from Korean sorghum (*Sorghum bicolor* L. Monech) [85]. Alloxan induces hypercholesterolemia in diabetics rats, and therefore, evident hyperlipidemia, which shows diabetic state, can be the consequence of inhibition of lipolytic hormones and thus decrease in catalysis of fat deposits [86]. The level of HDL cholesterol, total cholesterol triglycerides, LDL and VLDL cholesterol was considerably recovered by methanolic extract of *Sorghum halepense* (L.) Pers (Table 6) which shows that it demonstrates hypolipidemic characteristics. The inhibition of the synthesis of fatty acid may lead to lower the level of lipid. Normally insulin triggers lipoprotein lipase which hydrolyses triglycerides whereas in diabetic the owing to a shortage of insulin, inactivation of the mentioned enzyme may result in hypertriglyceridemia [87]. Following the treatment with *Sorghum halepense* (L.) Pers extract, a remarkable drop-off in serum lipid level in diabetic rats can be directly related to the restoration of insulin level.

Analogous findings were achieved during the antidiabetic study of Sorghum and *Galium tricornutum* extracts [81, 88].

An enzymatic antioxidant protection mechanism can be decreased with an increase in the concentration of lipid peroxidation [89]. In previous studies, the formation of oxygen free radicals in diabetic β cells and its deleterious effects have been documented. The mentioned cells can be saved from oxidative reparation by overexpression of antioxidant enzymes like SOD and CAT [82, 90, 91]. In the liver tissues, significant improvement was observed in the activities of SOD and CAT after treatment with the extract (150 and 300 mg/kg b.w.) during the current study. This recommends that the oxidative stress in diabetes was reduced by the applied extract due to its efficient antioxidant characteristics [90] and can enhance the activities of CAT and SOD [82, 92].

Dysfunction of β-cells and insulin resistance are key markers of diabetes [93] and the later one has a close relationship with altered lipid profile and serves as the major constituent of other metabolic disorders besides diabetes. For example, insulin resistance has been shown to be concerned with diabetic dyslipidemia characterized by high level of VLDL, TG, total cholesterol and low level of HDL [94, 95]. Consequently, the lipid profile is considered in almost all follow-up programs of diabetes and maybe useful for early intervention and hampering the progression of diabetes [93, 96]. After treatment with RSH methanolic extracts, a significant decline in the levels of lipid, ALT and bilirubin in the serum of diabetic rats can be directly related to the restoration of insulin concentration and thereby reduction in blood glucose level. Evaluation of medicinal plants with an aim to find new compounds having therapeutic activities such as antioxidants, hypolipidemic and antidiabetic [97, 98] is an emerging research area.

Conclusion

The current research project suggests that the rhizomes of *Sorghum halepense* (L.) Pers exhibit significant cytotoxic, antioxidant and anti-diabetic characteristics. The methanolic extract contained higher total phenolic contents than its fractions. Besides, total phenolic contents indicated a significant correlation with antioxidant activities (DPPH, ABTS and H_2O_2) while non-significant with antidiabetic activity. Finally, it is concluded that the extract of rhizomes of *Sorghum halepense* (L.) Pers is a valuable source of antioxidant and anti-diabetic compounds. Its further analysis is needed to sharpen its pharmacological activities.

Abbreviations

ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; HDL: High density lipoprotein; TG: Triglycerides; LDL: Low density lipoprotein; VLDL: Very low density lipoprotein; SOD: Superoxide dismutase; MDA: Malondialdehyde; SDS: sodium dodecyl sulfate; TBA: Thiobarbituric acid; TBARS: Thiobarbituric acid reactive substances; GSH: Glutathione; TCA: Trichloroacetic acid; EDTA: Ethylenediamine tetraacetic acid; DTNB: 5, 50-dithiobis-2-nitrobenzoic acid; NBT: Nitro blue tetrazolium

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Disclosure statement

The author declares that he/she has no conflict of interests.

Authors' contributions

MAS collected plant, conducted experiments, collected and analyzed data and drafting of the manuscript. RAK (ORCID ID: 0000–0003–0453-2090) and MA has made significant influence to onset and design, analysis of data and drafting of manuscript. The authors read and approved the final manuscript.

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Ethics approval and consent to participate

The study was conducted according to the protocol approved by Institutional Animals Ethics Committee (Ref. No. University of Science and Technology Bannu/ Biotech/Ethical/123) of the University.

Competing interests

The authors declare that they have no competing interests.

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