Antidiabetic antihyperlipidemic and hepato-protective effect of Gluconorm-5: A polyherbal formulation in steptozotocin induced hyperglycemic rats

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ABSTRACT

Background: The antidiabetic, antihyperlipidemic, and hepato-protective effect of Gluconorm-5, was studied in steptozotocin (STZ) induced hyperglycemic rats.

Materials and Methods: The hypoglycemic effect of single dose of Gluconorm-5 (150, 300 and 600 mg/kg body weight) made up of five plants namely *Camellia sinensis, Punica granatum, Macrotyloma uniflorum, Foeniculum vulgare and Trigonella foenum-graecum* was studied in normal, glucose loaded normal and diabetes-induced rats. The extent of antihyperlipidemic and liver-protective effect was studied by estimating the lipid profile, and the liver marker enzymes. Histopathological studies of the pancreatic tissue were also carried out with glibenclamide as standard antihyperglycemic agent.

Results: Fifteen days of oral feeding of the Gluconorm-5 (300 and 600 mg/kg) to diabetic rats resulted in a significant ($P < 0.01$) reduction of blood glucose, lipid profile, liver weight and marker enzymes as compared to those rats in whom STZ induced toxicity was untreated. The diabetic rats treated with the drug showed expanded islets as compared to the untreated diabetic rats, which showed the shrunken islets. The animals that received 300 mg/kg of Gluconorm-5 showed pronounced antidiabetic, antihyperlipidemic and hepato-protective effect in the present study, which was comparable with glibenclamide, a standard drug.

Conclusion: Gluconorm-5 exerts potent antidiabetic antihyperlipidemic and hepato-protective effect, which can be used as adjuvant in the treatment of diabetes mellitus.

KEY WORDS: Antidiabetic, antihyperlipidemic, Gluconorm-5, hepato-protective

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycemia because the body doesn't produce enough insulin, or cells don't respond to the insulin that is produced, with disturbances of carbohydrate, fat and protein metabolism.[1] The disease is very often associated with a marked increase in cardiovascular risk parameters such as hypertriglyceridemia and dyslipidemia. At present, the treatment of diabetes mainly involves a sustained reduction in hyperglycemia by the use of biguanides, thiazolidinediones, sulfonylureas, D-phenylalanine derivatives, meglitinides and α-glucosidase inhibitors in addition to insulin. However, due to unwanted side-effects, the efficacies of these compounds are debatable and there is a demand for new compounds for the treatment of diabetes.[2] WHO has recommended the evaluation of traditional plant treatments for diabetes as they are effective, nontoxic, with lesser or no side-effects and are considered to be excellent candidates for oral therapy.[3] Many Indian plants have been investigated for their beneficial use in different types of diabetes and reports occur in many scientific journals.[4]

In the Ayurvedic classics such as Caraksaṃhitā, Mādhava Nidāna and Aṣṭāṅga saṅgraha there are about 600 plants which are stated to have antidiabetic property.[5] There are many Indian plants, which are effective and commonly studied in relation to diabetes and its associated complications.[6,7] A single drug cannot be effective against severe diseases. Therefore, there is a need to develop effective formulations using indigenous medicinal plants, subjecting them to pharmacological experiments and clinical trials. In the present study, a polyherbal formulation namely Gluconorm-5 containing medicinal plants as shown in

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Table 1 was subjected to various assays in order to evaluate its antidiabetic activity. These plants have been used traditionally for the treatment of diabetes mellitus and all of them are scientifically evaluated for their potency individually.[8-11] The intention of the present study is to scrutinize the effect of oral administration of Gluconorm-5 on the blood glucose levels and to ascertain the scientific basis for the use of this polyherbal extract in the management of Type-II diabetes mellitus using steptozotocin (STZ) induced diabetic rats. The objective of this study was also to study whether Gluconorm-5 is effective in reducing the cardiovascular complications associated with diabetes mellitus.

MATERIALS AND METHODS

Chemicals

All chemicals used in the study were obtained from SD Fine Chemicals, Mumbai. STZ and glibenclamide were obtained from Sigma chemicals Bangalore. All the chemicals used were of analytical grade.

Collection of plant material

The medicinal plants composing Gluconorm-5 formulation were collected from Koyambedu market and were authenticated by Dr. Sankaranarayanan, Assistant Director, Department of Research and Development, Sairam Siddha Medical College and Research Centre, Chennai, India. The voucher specimen is also available in herbarium file of the same center.

Preparation of Gluconorm-5 extract

The parts of the plant as mentioned in Table 1 were shade-dried and pulverized to a coarse powder. Equal quantities (25 g each) of the plant powder was passed through 40-mesh sieve, mixed and exhaustively extracted with 90% (v/v) ethanol in Soxhlet apparatus at 60°C. The extract was evaporated under pressure till all the solvent had been removed. Further removal of water was carried out by freeze drying which led to an extract sample with yield of 12.56% (w/w). The extract was stored in the refrigerator and a weighed amount of the extract was dissolved in 2% (v/v) aqueous Tween-80 and used for the present investigation.

Table 1: Composition of the Gluconorm-5 extract

Botanical name of plant	Family	Parts used	Common name
Camellia sinensis	*Theaceae*	Leaves	Green tea
Punica granatum	*Punicaceae*	Outer rind	Pomegranate
Macrotyloma uniflorum	*Fabaceae*	Seeds	Horse gram
Foeniculum vulgare miller	*Apiaceae*	Seeds	Fennel
Trigonella foenum-graecum	*Fabaceae*	Seeds	Fenugreek

Animals

Adult albino male rats of Wistar strain weighing 200–250 g were used in the pharmacological and toxicological studies. The inbred animals were taken from animal house in Vel's College of Pharmacy, Chennai, India. The animals were maintained in well ventilated, room temperature conditions with natural 12 ± 1 h day–night cycle in the propylene cages. They were fed balanced rodent pellet diet from Poultry Research Station, Nandanam, Chennai, India and tap water *ad libitum* was provided throughout the experimental period. The animals were sheltered for 1-week prior to the experiment and were acclimatized to laboratory temperature. The protocol was approved by Animal Ethics Committee constituted for the purpose as per CPCSEA Guideline.

Acute toxicity studies

Acute toxicity studies were conducted with the Gluconorm-5 extract in Wistar albino rats by staircase method.[12] Albino rats of either sex were selected and segregated into 8 groups of 6 animals each. Single dose of Gluconorm-5 extract dissolved in 2% aqueous Tween 80, starting from the minimal dose of 50 mg/kg up to 3000 mg/kg were administered orally. The animals which were administered with the drug-treated animals were observed carefully for toxicity signs and mortality. LD_{50} doses were selected for the evaluation of antihyperglycemic activity. All the animals were also observed for further 14 days for various clinical symptoms and mortality.

Hypoglycemic activity screening of Gluconorm-5 in normal rats

The normal albino rats were first used for screening the hypoglycemic activity of the Gluconorm-5 formulation according to the method adapted by Skim *et al*.,[13] and Sabu *et al*.,[14] with slight modifications. Overnight fasted normal rats were randomly divided into five groups with six animals in each group. Five groups received single dose of either 1.0 ml vehicle (2% v/v aqueous Tween 80), Gluconorm-5 (150, 300 and 600 mg/kg) or glibenclamide 1.0 mg/kg orally. Blood samples were collected from the tail vein prior to and 30, 60, 90 and 120 min after treatment. Fasting blood glucose and fall in the blood glucose were calculated by using one touch glucometer.

Effect of Gluconorm-5 on glucose tolerance in normal rats

The effect of the Gluconorm-5 extract on glucose tolerance was studied by the method of Williamson *et al*.[15] Overnight fasted normal rats were randomly divided into five groups with six animals in each group and were given the medicine according to a dose schedule as follows: Group I: Animals were given

a single administration of 1.0 ml vehicle (2% v/v aqueous Tween 80) po. This group served as control. Groups II, III and IV: Animals were treated orally a single dose of Gluconorm-5 with doses of 150, 300, 600 mg/kg respectively in the vehicle. Group V: Animals received glibenclamide 1.0 mg/kg orally. The rats of all groups were given glucose (1 g/kg body weight, p.o) 30 min after administration of the drug. Blood samples were collected from the tail vein just prior to glucose administration and at 30, 60, 90 and 120 min after the glucose loading and blood glucose levels were measured immediately by using One touch glucometer.

Induction of experimental diabetes

Overnight fasted albino rats ($n = 45$) were made diabetic by injecting with freshly prepared solution of STZ (60 mg/kg in 0.01 M citrate buffer, pH - 4.5) an effective agent for induction of diabetes mellitus.[16] The diabetic state was assessed in STZ treated rats by measuring the nonfasting plasma glucose concentration after 48 h. Only rats with plasma glucose level greater than 300 mg/dl were selected and used in this experiment.

Experimental protocol for the evaluation of the hypoglycemic activity of the Gluconorm-5

The rats were divided into 5 groups of 6 rats and were given a dose schedule as follows: Group I: Served as normal healthy controls and Group II STZ treated diabetic rats were given a single administration of 0.5 ml vehicle (2% v/v aqueous Tween 80) p.o. for 15 days. Groups III and IV consisted of STZ treated diabetic rats which received Gluconorm-5 at a dose of 300 and 600 mg/kg p.o. respectively in vehicle for 15 days. Group V consisted of diabetic rats which received glibenclamide 1 mg/kg p.o. Group VI consisted of normal healthy animals receiving 300 mg/kg p.o of Gluconorm-5 for 15 days. Fasting blood samples were collected from the tail vein for blood glucose estimation on 0, 4, 8 and 15th day using one touch Glucometer. The food and water intake was monitored daily for each rat, and the periodical body weight difference of the individual animals was also measured during 15 days of the experimental period.

On the 15th day, the animals were sacrificed by cervical decapitation and various biochemical parameters were analyzed.

Biochemical analysis

At the end of the experimental period, overnight fasted animals were sacrificed by cervical decapitation under light ether anesthesia and blood was collected, serum was separated by centrifuging at 3,000 rpm for 10 min. The serum was used for the assay of the biochemical parameters such as total cholesterol (TC), High-density lipoprotein-cholesterol (HDL-C), Low-density lipoprotein-cholesterol (LDL-C) cholesterol and triglycerides using the diagnostic kits. The liver marker enzymes, such as alanine aminotransferase (ALT),[17] aspartate aminotransferase (AST),[17] alkaline phosphatase (ALP)[18] and lactate dehydrogenase (LDH)[19] were also estimated. Liver and pancreas were excised from the animals, washed in ice-cold saline, and dried gently on the filter paper. The weight of the liver was taken. Liver glycogen[20] was estimated. All the enzymatic and biochemical assays were read at specific wavelength using Shimadzu spectrophotometer, UV-1601 model shimadzu corporation, Japan.

Histopathological investigations

The dissected samples of pancreas from each group of diabetic animals were collected in 10% formalin-saline solution and stained with hemotoxylin and eosin for preparation of section using a microtome and histopathological studies were carried out.

Statistical analysis

Values reported are mean ± standard error. The statistical analysis was carried out using analysis of variance, followed by Dunnet's *t*-test. $P < 0.05$ were considered as significant.

RESULTS

Acute toxicity studies

No toxic symptoms were observed after administration of different dose levels of extract up to a maximum of 3000 mg/kg p.o. according to OECD guideline 423. In addition to this, a dose of 5000 mg/kg dose was administered to a group of animals and symptoms like dyspnea were identified. Hence, the one tenth and one-twentieth of safe, tolerable dose was used as a therapeutic dose for further pharmacological study. From this experiment, the therapeutic dose level of Gluconorm-5 was fixed as 300 mg/kg. The double multiple of this dose, of 600 mg/kg was also considered for comparing the effectiveness of the maximum therapeutic dose, in the antidiabetic and the antihyperlipidemic activity of STZ induced diabetic rats.

Hypoglycemic activity screening of Gluconorm-5 in normal rats

Table 2 shows the hypoglycemic activity of the Gluconorm-5 in normal rats. The onset of hypoglycemic activity of Gluconorm-5 at 150, 300 and 600 mg/kg was evident

between the 60 and 90 min after the administration of the drug. Among the three concentrations significant ($P < 0.05$) hypoglycemic activity was observed in the Groups III and IV animals, which received Gluconorm-5 at the concentration of 300 mg and 600 mg/kg body weight. In these concentrations, the drug exhibited pronounced hypoglycemic activity than the reference drug glibenclamide.

Effect of Gluconorm-5 on Glucose tolerance in normal rats

The plasma glucose level of the control animals reached a peak (256 mg/dl) at 60 min after the oral glucose (1 g/kg) administration and gradually decreased (140 mg/dl) at 120 min [Table 3]. In the present investigation the Gluconorm-5 treated (150, 300 and 600 mg/kg body weight) rats have shown significant ($P < 0.01$, $P < 0.05$) increase in glucose tolerance in comparison to the control group rats. Drug treatment has considerably reduced the onset and effect of peak blood glucose levels. The reduction of blood glucose level is more pronounced in Groups III and IV (300 and 600 mg/kg treated) rats than Group II (150 mg/kg treated) rats. The blood glucose levels were found to be normal in these drugs treated groups.

Antihyperglycemic activity of Gluconorm-5 in experimentally induced diabetic rats

There was a significant reduction of blood glucose level in Group III and Group IV diabetic rats which received Gluconorm-5 (300 mg/kg and 600 mg/kg body weight) as compared ($P < 0.001$) with STZ induced untreated diabetic Group II rats [Table 4]. In the present investigation it was observed that these extracts were able to reduce the blood glucose level from the day-1 of its administration

Table 2: Effect of Gluconorm-5 on blood glucose level in normal fasted rats

Treatment	Blood glucose level (mg/dl)				
	Fasting	30 min	60 min	90 min	120 min
Group I (control)	69.6±1.45	67.8±1.39	66.7±1.36	63.5±1.85	60.8±1.67
Group II Gluconorm-5 (150 mg/kg)	71.6±2.37a*	69.5±1.27a*	55.8±1.53a*	52.7±1.23a*	60.5±1.37a*
Group III Gluconorm-5 (300 mg/kg)	68.9±1.15b**	66.5±1.35b**	50.6±2.14b**	51.6±0.85b**	58.2±1.14b**
Group IV Gluconorm-5 (600 mg/kg)	67.9±1.33c**	66.9±1.15c**	54.4±1.45c**	53.6±1.29c**	56.7±1.24c**
Group glibenclamide (1 mg/kg)	74.7±1.28	69.3±2.45	62.6±0.95	60.7±1.67	59.8±1.89

*$P<0.05$, **$P<0.01$, ***$P<0.001$. Values are mean±SEM from 6 animals in each group. Statistical significant test for comparison was done by ANOVA, followed by *post-hoc* Dunnet's *t*-test. Comparison between: a-Group I versus Group II, b-Group I versus Group III, c-Group I versus Group III. NS: Nonsignificant, SEM: Standard error of mean

Table 3: Effect of Gluconorm-5 on blood glucose level in glucose loaded rats

Treatment	Blood glucose level (mg/dl)				
	Fasting	30 min	60 min	90 min	120 min
Group I (control)	74.0±2.8	172.4±2.2$^{\#\#\#}$	256.7±1.36$^{\#\#\#}$	238.3±5.9$^{\#\#\#}$	140.8±1.37$^{\#\#\#}$
Group II Gluconorm-5 (150 mg/kg)	77.56±1.8a*	153.7±3.0a*$^{\#\#}$	195.8±1.13a*$^{\#\#}$	162.7±1.23a*$^{\#\#}$	90.5±1.43a*$^{\#}$
Group III Gluconorm-5 (300 mg/kg)	79.2±2.1b**	92.1±4.6b**$^{\#}$	181.6±2.60b**$^{\#\#\#}$	83.8±3.0b**$^{\#}$	78.2±1.11b**^{ns}
Group IV Gluconorm-5 (600 mg/kg)	75.6±2.0c*	83.4±2.1c*$^{\#}$	168.4±1.35c*$^{\#\#}$	82.1±2.2b*$^{\#}$	76.7±1.20c*^{ns}
Group V glibenclamide (1 mg/kg)	73.4±2.4d^{ns}	69.3±2.45d**	162.6±0.95d**	90.7±1.67d***	69.8±1.19d**

*P values are statistically significant when compared with normal, $^{\#}P$ values are statistically significant when compared with time, *$^{\#}P<0.05$, **$^{,\#\#}P<0.01$, ***$^{,\#\#\#}P<0.001$. Values are mean±SEM from 6 animals in each group. Statistical significant test for comparison was done by ANOVA, followed by *post-hoc* Dunnet's *t*-test. Comparison between: a-Group I versus Group II, b-Group I versus Group III, c-Group I versus Group III. NS: Nonsignificant, SEM: Standard error of mean

Table 4: Effect of Gluconorm-5 on blood glucose level in different groups of experimental rats

Treatment	Blood glucose level (mg/dl)				
	Day 1	Day 4	Day 8	Day 12	Day 15
Group I (control)	66.45±2.88	71.40±3.67	73.80±2.98	72.45±1.23	73.4±2.90
Group II STZ	445.55±13.40a***	458.1±12.4a***	465.32±10.12a***	467.78±12.34a***	460.45±5.68a***
Group III STZ + Gluconorm-5 (300 mg/kg)	70.12±3.11b***	68.2±2.40b***	65.63±3.00b***	66.34±1.89	66.11±2.22b***
Group IV STZ + Gluconorm-5 (600 mg/kg)	71.60±2.66c***	72.33±2.30c***	72.12±2.37c***	75.29±2.87	74.33±2.60c***
Group V STZ + glibenclamide (1 mg/kg)	90.55±2.65	100.00±3.36	94.2±4.88	96.36±3.45	101.18±4.33
Group VI Gluconorm-5 (300 mg/kg)	70.10±1.36d^{ns}	69.33±1.68d^{ns}	68.40±1.26d^{ns}	70.12±2.13	62.4±2.30d^{ns}

*$P<0.05$, **$P<0.01$, ***$P<0.001$. Values are mean±SEM from 6 animals in each group. Statistical significant test for comparison was done by ANOVA, followed by *post-hoc* Dunnet's *t*-test. Comparison between: a-Group I versus Group II, b-Group II versus Group III, c-Group II versus Group IV, d-Group I versus Group VI. NS: Nonsignificant, SEM: Standard error of mean, STZ: Steptozotocin

indicating the strong hypoglycemic tendency of the phyto-constituents present in the extract. A sustained and significant ($P < 0.001$) decrease in the blood glucose level is observed throughout the experimental period in the rats treated with Gluconorm-5 extract. Significant hypoglycemic activity ($P < 0.001$) is observed in the Group III animals, which received 300 mg/kg when compared to 600 mg/kg dose level. The blood glucose lowering potential of the test drugs is comparable to that of standard drug glibenclamide, which was used as a positive control in the present study.

Effect of Gluconorm-5 on Body weight and liver weight

The effect of Gluconorm-5 on body weight and liver weight in various experimental rats has been shown in Table 5. It was observed that the body weight of STZ induced Group II diabetic animals gradually decreased when compared ($P < 0.05$) to the normal rats indicating the impaired glucose metabolism. However, the drug-treated Group III and Group IV animals maintained the body weight, which indicates the drug treatment has ameliorated the impaired carbohydrate metabolism. In the present investigation, a significant ($P < 0.01$) reduction in the liver weight was shown in the Gluconorm-5 treated Groups III and IV animals when compared to that of streptozocin toxin induced diabetic untreated Group II rats showing the liver protective role of the plant extract.

Effect of Gluconorm-5 on serum lipid profile

The serum lipid profile of the control and the experimental animals has been depicted in Table 6. The concentration of triglycerides and cholesterol were significantly increased in Group II diabetic animals when compared ($P < 0.001$,) to drug-treated Group III and Group IV animals. The increased levels of cholesterol and triglycerides were brought back to near normal by the treatment with Gluconorm-5. This observed restoration of the STZ evoked changes in the serum lipid profile shows the protective nature of Gluconorm-5. In the present investigation, there is a considerable increase in the HDL-C level ($P < 0.05$) in the animals which received the test drugs when compared to control animals.

Effect of Gluconorm-5 on marker enzymes and liver glycogen

Table 7 depicts the change in serum levels of the liver marker enzymes like AST, ALT, ALP and LDH. A significant increase in the serum transaminases AST and ALT levels was seen in the STZ induced diabetic untreated Group II animals. These enzymes were brought back to near normal levels in Gluconorm-5 treated Group III and Group IV animals ($P < 0.01$). Similarly the elevated ALP and LDH enzyme levels in toxin induced Group II animals were

Table 5: Effect of Gluconorm-5 on body weight and liver weight changes in different groups of experimental rats

Treatment	Periodical weight changes in grams after Gluconorm-5 treatment					Liver weight (mg/g body weight)
	Day 1	Day 4	Day 8	Day 12	Day 15	Day 15
Group I (control)	232.0±7.4	240.0±8.0	246.0±7.2	260.0±9.1	273.0±8.5	38.67±0.41
Group II STZ	230.0±8.5	230.0±10.2	208.0±4.0	208.0±9.0a*	182.0±8.0a**	45.34±0.41a*
Group III STZ + Gluconorm-5 (300 mg/kg)	232.0±12.0	233.0±15.0	236.0±18.0	243.0±12.0b*	243.0±6.0b**	35.56±0.24b**
Group IV STZ + Gluconorm-5 (600 mg/kg)	224.6±5.5	222.5±7.5	234.0±4.0	236.5±5.2c*	235.0±5.0c*	36.16±0.54c**
Group V STZ + glibenclamide (1 mg/kg)	227.2±12.0	228.0±14.0	232.0±17.7	238.0±12.6	238.0±15.2	33.56±0.17
Group VI Gluconorm-5 (300 mg/kg)	230.3±10.1	231.0±14.1	230.9±16.1	228.8±10.1	229.4±17.1d**	36.56±0.68d^{ns}

*$P<0.05$, **$P<0.01$, ***$P<0.001$. Values are mean±SEM from 6 animals in each group. Statistical significant test for comparison was done by ANOVA, followed by *post-hoc* Dunnet's *t*-test. Comparison between: a-Group I versus Group II, b-Group II versus Group III, c-Group II versus Group IV, d-Group I versus Group VI. NS: Nonsignificant, SEM: Standard error of mean, STZ: Steptozotocin

Table 6: Effect of Gluconorm-5 on serum lipid profile in different groups of experimental rats

Treatment	Parameters (mg/dl)				
	TC	HDL-C	LDL-C	VLDL-C	TG
Group I (control)	125.33±3.67	53.36±5.5	53.67±2.90	17.64±1.6	88.2±2.71
Group II STZ	192.30±4.54a**	31.56±2.43a**	112.45±1.33a**	48.68±3.3a**	125.4±6.00a**
Group III STZ + Gluconorm-5 (300 mg/kg)	124.60±3.0b**	48.63±3.97b*	71.45±3.47b**	14.4±2.67b**	68.0±2.14b**
Group IV STZ + Gluconorm-5 (600 mg/kg)	125.31±5.5c**	43.63±3.97c*	68.34±2.56c**	13.78±3.33c**	70.0±2.10c**
Group V STZ + glibenclamide (1 mg/kg)	118.34±4.3	51.37±2.41	50.45±1.98	16.78±1.45	85.2±3.35
Group VI Gluconorm-5 (300 mg/kg)	113.89±7.2d*	41.25±3.6d*	58.23±1.67d*	15.58±1.34d*	75.4±1.44d*

*$P<0.05$, **$P<0.01$, ***$P<0.001$. Values are mean±SEM from 6 animals in each group. Statistical significant test for comparison was done by ANOVA, followed by *post-hoc* Dunnet's *t*-test. Comparison between: a-Group I versus Group II, b-Group II versus Group III, c-Group II versus Group IV, d-Group I versus Group VI. NS: Nonsignificant, SEM: Standard error of mean, STZ: Steptozotocin, TC: Total cholesterol, HDL-C: High-density lipoprotein cholesterol, TG: Triglycerides, LDL-C: Low-density lipoprotein cholesterol, VLDL-C: Very-low-dHTC ensity lipoprotein

Table 7: Effect of Gluconorm-5 on marker enzymes and liver glycogen in different groups of experimental rats

Treatment	AST (U/L)	ALT (U/L)	LDH (U/L)	ALP (IU/L)	Liver glycogen (mg/g wet tissue)
Group I (control)	33.78±4.7	34.23±3.7	145.90±1.87	76.66±0.53	55.78±3.23
Group II STZ	113.79±4.50a**	115.50±1.08a**	298.05±4.70a***	173.16±2.29a***	21.56±2.78a***
Group III STZ + Gluconorm-5 (300 mg/kg)	77.30±3.40b**	82.11±2.45b**	234.56±6.98b**	126.57±5.82b***	39.67±3.16b**
Group IV STZ + Gluconorm-5 (600 mg/kg)	76.92±3.60c**	88.75±1.46c**	255.69±7.34c**	132.48±2.24c***	36.98±4.31c**
Group V STZ + glibenclamide (1 mg/kg)	76.92±3.60	79.75±2.06	225.38±5.90	120.16±3.93	40.69±3.12
Group VI Gluconorm-5 (300 mg/kg)	35.75±1.46d^{ns}	39.78±1.89d^{ns}	148.67±3.67d^{ns}	80.34±1.39d^{ns}	56.45±2.90d^{ns}

*P<0.05, **P<0.01, ***P<0.001. Values are mean±SEM from 6 animals in each group. Statistical significant test for comparison was done by ANOVA, followed by *post-hoc* Dunnet's *t*-test. Comparison between: a-Group I versus Group II, b-Group II versus Group III, c-Group II versus Group IV, d-Group I versus Group VI. NS: Nonsignificant, SEM: Standard error of mean, STZ: Steptozotocin, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, LDH: Lactate dehydrogenase, ALP: Alkaline phosphatase

also significantly decreased in the drug treated and STZ toxin induced animals ($P < 0.01$, $P < 0.001$). Drug treatment reverted the increased levels of these enzymes to near normalcy ($P < 0.001$) which was comparable to that of a standard drug glibenclamide used in the present study. The animals that received STZ alone showed decrease in the glycogen level compared to ($P < 0.001$) control animals. Administration Gluconorm-5 (300 and 600 mg/kg body weight) and glibenclamide (1 mg/kg body weight) restored the liver glycogen significantly and the values were close to the normal values. Comparison of Group I control rats with that of Group VI rats, which received only Gluconorm-5 showed no significant variation in the marker enzyme levels and liver glycogen indicating no adverse side-effects due to the administration of Gluconorm-5 alone.

Histopathological studies

The photomicrograph of vehicle-treated nondiabetic control rats showed normal acini and normal cellular population of islets of β-cells. The islets of STZ induced diabetic rats showed extensive necrotic changes, followed by atrophic acini and reduction of β-cell size [Figure 1].

The Gluconorm-5 (300 and 600 mg/kg body weight) treated animals showed minimal to moderate degree of necrotic changes with marked proliferated and regenerated β-cells. The standard drug glibenclamide treated Group V animals showed the hyperplastic condition of β-cells. No significant changes were observed in Group VI the Gluconorm-5 alone treated animals, and the normal architecture of cells is been protected indicating the absence of adverse side effects of the formulation.

DISCUSSION

Diabetes mellitus is a syndrome, initially characterized by loss of glucose homeostasis resulting from defects in insulin secretion and insulin action both resulting impaired metabolism of glucose and other energy-yielding fuels such as lipids and protein.[21] In the traditional system of Indian medicine, plant formulation and combined extracts of plants are used as drugs of choice rather than individual entities. Various herbal formulations such as diamed,[22] coagent db[23] and hyponid,[24] are well known for their antidiabetic effects. Experimental diabetes in animals has provided considerable insight into the physiological and biochemical derangement of the diabetic state. Significant changes in lipid metabolism and structure also occur in diabetes.[25] The Gluconorm-5 is composed of five medicinal plants [Table 1], which are known and already reported for their antidiabetic activity.

In the present study, significant sugar lowering activity was observed in the Group III animals which received Gluconorm-5 at the concentration of 300 mg/kg body weight. In this group the onset of hypoglycemic activity was evident between the 60 and 90 min after the administration of the drug. The glucose lowering potential of the herbal drug Gluconorm-5 was more than that of the reference drug glibenclamide used in the present study. The presence of many active principles such as dietary fibers, alkaloids, flavonoids, saponins, amino acids, steroids, peptides and others phyto chemicals present in the Gluconorm-5 may be responsible for the potent hypoglycemic and glucose suppressive activities.[26]

The Glucose tolerance test measures the effect of plant extracts in reducing blood glucose in normoglycemic condition. In the present investigation, the glucose tolerance of the Gluconorm-5 treated rats increased significantly. The glucose lowering effect of the Gluconorm-5 may be due to the effect of active constituents of different plants viz. trigonelline, scopoltin, alkaloid-6-methoxybenzoxazolinone, terpenoids such as scoparic acids A, B, C and scopadulcic acid A and B from *Trigonella foenum graecum*,[27] polyphones such as catechin, epicatechin, epigallocatechin, and their gallates, teanin and caffeine from *Camellia sinensis*,[28] essential oils containing spetroselinic acid, oleic acid, trans-anethole,

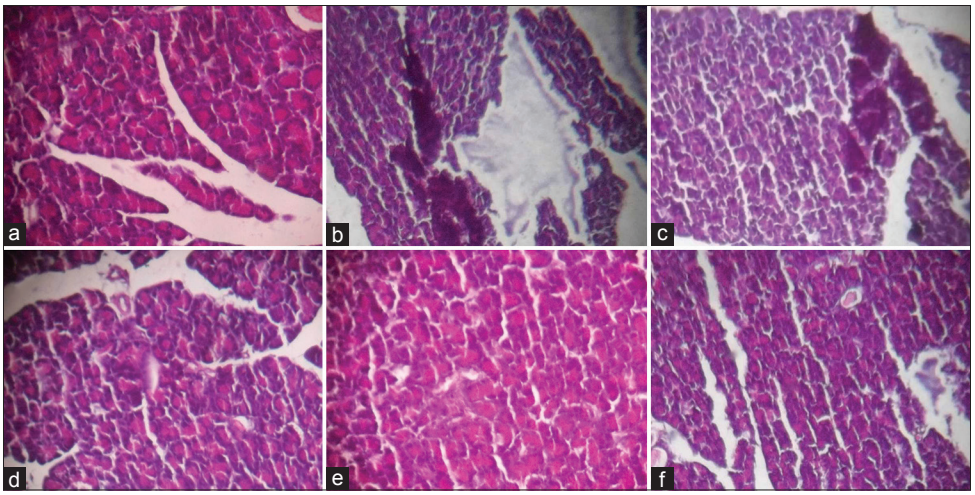

Figure 1: Histopathological changes which have occurred in the pancreas after steptozotocin intoxication and protection by treatment with Gluconorm-5 (H and E, ×400). (a) Nondiabetic control with normal acini with islets of β-cells; (b) Diabetic control with atrophic acini and reduction of β-cell size; (c) Gluconorm-5 (300 mg/kg) treated cells with markedly normal regenerated and preserved cells; (d) Gluconorm-5 (600 mg/kg) treated cells with proliferated and regenerated β-cells; (e) Glibenclamide treated cells with hyperplastic condition; (f) Gluconorm-5 alone treated cells showing normal architecture of β-cells

fenchone of limonene, camphor, alpha-pinene, and tocopherol of fennel seeds.[29] The reduced blood glucose levels were found to be normal in these drugs treated groups.

Steptozotocin is a nitrosurea compound produced by *Streptomyces achromogenes*, which specifically induces DNA strand breakage in ß-cells resulting in a decrease in endogenous insulin release, which paves the ways for the decreased utilization of glucose by the tissues causing diabetes mellitus. Therefore, the STZ-diabetic model has been widely employed to induce diabetes in experimental animals.[30] In the present investigation a significant reduction in the blood glucose was shown in Gluconom-5 treated Group III and Group IV animals when compared to that of Group II STZ induced diabetic animals from the day-1 of its administration. The antihperglycemic activity in this condition is due to the presence of the biomolecules of plant origin present in the herbal formulation. This may be achieved either by the restoration and regeneration of pancreatic cells thereby increasing the insulin secretion which decreases blood glucose level, or by decreasing the glucose absorption in the gut by inhibiting the effect of key enzymes related to starch digestion and its absorption. Earlier studies have reported that administration of herbal terpenes such as b-pinene, existing in fenugreek oil protects the architecture of pancreatic β cells, preserves the insulin secretion and stimulates the regeneration of this type of cells.[31,32]

In the present investigation, it was observed that the body weight of STZ induced Group II diabetic animals gradually decreased when compared to the normal rats indicating the impaired glucose metabolism. Induction of diabetes by STZ leads to loss of body weight due to increased muscle wasting and loss of tissue proteins.[33] After 15 days of Gluconorm-5 treatment, gain in body weight was recognized in diabetic rats, and the results were comparable with that of the standard drug, glibenclamide. The phytochemicals in the herbal drug might enhance the glucose utilization and improve the diabetes-associated complications thereby restoring the body homeostasis and increasing the body weight. A significant increase in liver weight was observed in STZ induced diabetic rats due to the hepatocyte injury and lipid peroxidation of the liver cells. The STZ causes CYP2E1 dependent oxidative stress and damage the liver cells. The drug treatment significantly reduced the liver weight and brought back to near normalcy indicating the hepato protective nature of the Gluconorm-5.

Apart from the regulation of carbohydrate metabolism, insulin also plays an important role in the metabolism of lipids. It is evident in the present investigation that STZ induced diabetic rats exhibited hypercholesterolemia, hyper triglyceridemia and low level of HDL. According to Bierman *et al*.,[34] abnormal increase of blood glucose in diabetes mellitus is associated with dyslipidemia in both clinical and experimental diabetes. Repeated administration of Gluconorm-5 led to the significant reduction in TC, triglycerid (TG) and LDL-C with a concomitant increase in the HDL-C indicating the potential role of the extract in correcting the dyslipidemic

condition associated with diabetes mellitus. Insulin is a potent inhibitor of lipolysis by inhibiting the activity of the hormone sensitive lipases and suppresses the release of free fatty acids from the adipose tissue.[35] During diabetes, enhanced activity of this enzyme increases lipolysis and releases more free fatty acids into the circulation which in turn increases the β-oxidation of fatty acids, producing more acetyl-CoA and cholesterol. In normal condition, insulin increases the receptor-mediated removal of LDL-C and due to insulin deficiency the serum level of LDL-C increases leading to hypercholesterolemia.[36] Reduction in the production of HDL-C is due to the impaired metabolism of TG-rich lipoproteins, which provide a significant portion of HDL-C when metabolized by the enzyme lipoprotein lipase.[37] Dysfunction of lipoprotein lipase in insulin-deficient state also contributes to hypertriglyceridemia due to impaired catabolism of triglyceride rich particles.[38]

Punica granatum contains Anthocyanins, in the form of cyanidin-3-*O*-glucoside, cyanidin-3,5-di-*O*-glucoside, delphinidin-3-*O*-glucoside, delphinidin-3,5-di-*O*-glucoside, pelargonidin-3-*O*-glucoside, and pelargonidin-3,5-di-*O*-glucoside.[39] The phenolic acids present in pomegranate can be divided into two groups namely hydroxybenzoic acids, mainly gallic acid and ellagic acid.[40] and hydroxycinnamic acids, principally caffeic acid, chlorogenic acid, and *p*-coumaric acid[16,41] According to Kobayashi *et al*.,[42] the catechin and epigallocatechin-3-gallate (EGCG) which is found in the leaves of green tea was found to inhibit intestinal glucose uptake by the sodium-dependent glucose transporter SGLT1 thereby controlling the blood glucose level. Furthermore, the investigators showed that EGCG mimics insulin, increases tyrosine phosphorylation of the insulin receptor and the insulin receptor substrate, and reduces gene expression of the gluconeogenic enzyme phosphoenol pyruvate carboxykinase. Recently, green tea and green tea extracts were demonstrated to modify glucose metabolism beneficially in experimental models of type II diabetes mellitus by ameliorating cytokine-induced β cell damage *in vitro* and preventing the decrease of islet mass induced by treatment with multiple low doses of STZ *in vivo*.[43]

Al-Habori and Raman,[44] in their study showed that the alkaloids such as trigonelline, trigocoumarin, and trimecoumarine present in *T. foenum-graecum* possess antidiabetic and hypocholesterolemic effect and according to Gupta *et al*.,[45] and Sharma *et al*.,[46] the fibers and the guar gum of fenugreek seeds reduces the rate of glucose absorption and may also delay gastric emptying, thereby not only preventing the rise in blood sugar levels following a meal but also effective in the treatment of hypercholesterolemia. Moreover the amino acid, 4-hydroxyl isoleucine[47] of the seed fiber of *T. foenum-graecum* powerfully stimulates insulin secretion. The cumulative effect of above-mentioned active bio constituents is expected to be present in Gluconorm-5 and hence its ability to reduce increased blood glucose level and also correcting the dyslipidemic condition.

Hepatic glycogen content was found to be decreased in diabetic animals which were restored toward normal state in Gluconorm-5 treated rats. Conversion of glucose to glycogen in the liver cells is dependent on the extracellular glucose concentration and on the availability of insulin which stimulates glycogen synthesis over a wide range of glucose concentrations.[48] In the present investigation, Gluconorm-5 and glibenclamide treated rats showed the increased concentration of glycogen reserve indicating the phyto constituents present in the test drug have got the ability to stimulate the glycogen synthesis in the liver. STZ induces CYP2E1 dependent oxidative stress and causes hepatotoxicity.[49] When the hepatocytes get damaged, the intracellular enzymes, such as transaminases and serum ALP are released into the circulation after STZ administration due to the cellular damage.[50] The plasma levels of the liver marker enzymes such as AST ALT, ALP and LDH were significantly increased in STZ induced untreated Group II animals, and these enzyme levels were significantly reduced in Gluconorm-5 treated animals. Hence, it was possible that the active ingredients present in Gluconorm-5 may act against the oxidative stress related hepatic cellular injury produced by the CYP2E1 induction in type-1 diabetes and thereby protect the liver.

Histopathological examination of the pancreas also provided supportive evidence for this study. Pancreas of rats administered with STZ showed atrophic acini and reduction of β-cell size. Whereas the pancreas of the animals treated with Gluconorm-5 showed marked proliferated, and regenerated β-cells suggesting the possibility of test drug being able to condition the pancreatic cells towards accelerated regeneration.

CONCLUSION

Based on the above results, it can be concluded that Gluconorm-5, a combination of five herbal plants exerts significant antidiabetic, antihyperlipidemic and antiperoxidative effects. This could be due to different types of active principles, each with a single or a diverse range of biological activities. Gluconorm-5 may serve as a good

adjuvant in fighting diabetes. This herbal product can be used as an antidiabetic agent for human diabetic patients after completing the preclinical studies to ascertain its therapeutic efficacy and safety.

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