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Effect of *Terminalia chebula* fruit extract on antihyperglycemic and renal function markers in streptozotocin induced type 2 diabetic rats

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Abstract
The aim of this research was to estimate *Terminalia chebula* fruit anti-diabetic properties in Type 2 diabetic rats based on streptozotocin. Methanol extract of *Terminalia chebula* fruit significantly (p<0.05) decreased the level of fasting serum glucose. However, higher levels of liver glycogen and plasma insulin were seen in HFD / STZ controls relative to normal control animals and large increases of HbAlc rates at the end of the study. Besides *Terminalia chebula* hypoglycemic effect, a favorable effect was also found in the lipid profile. At the end of the study period, overall serum cholesterol and triglyceride were substantially reduced. The addition of *TCF* has substantially decreased in renal function markers (P<0.05) relative to the category HFD/STZ. Glibenclamide administration was also substantially decreased (p<0.01) in serum glucose levels. The experimental findings thus indicate that *Terminalia chebula* has hypolipidemic and hypoglycemic effects and may act as a source of strong antidiabetic drugs.

Keywords: Diabetes, *Terminalia chebula*, HFD, serum

Introduction

Worldwide the human race seems to be in the middle of a diabetes crisis. An approximate 285 million individuals suffer from diabetes, which amounts to 6.4 per cent of the population of the world. By 2030, the no. is projected to rise to four hundred and thirty eight million, which is 7.8 % of the adult population. Despite the tremendous advances made in identifying and treating diabetes, the problems connected with the condition and disorder remains growing unabated. Similar to this, recent advances in recognizing the disease process's pathophysiology have opened up many different opportunities for discovering and designing experimental treatments to fight the diabetic epidemic. At the same time, phytochemicals known from conventional medicinal plants offer an interesting possibility to create new forms of therapies. It has intensified the global attempt to harvest and harvest certain medicinal plants which carry considerable amounts of possible phytochemicals with numerous valuable effects in the fight against diabetes and difficulties associated with diabetes. Therefore, when the disease unabatedly advances, there is an immediate need to locate indigenous natural resources in order to acquire them and to research in depth their capacity for different newly defined targets in order to improve them.

Diabetes is a persistent condition in which insulin incorrectly controls homeostasis of the food, protein, and lipid metabolism. It contains elevated levels of fasting and post-prandial blood sugar. It is a chronic metabolic disease arising from shortage of insulin or deficiency of the hormone.

Insulin is a type of hormone essential in carbohydrate and body fat metabolism regulation. Its secretion triggers involve protein and glucose absorbed into the blood generated from digested food. Insulin attaches to the extracellular segment of the insulin receptor's alpha subunits, which in effect triggers a conformation shift in the insulin receptor that stimulates the kinase domain located on the beta subunit's intracellular part. It also triggers a chain of protein kinases and a whole sequence of phosphorylation/dephosphorylation enzyme reactions that compensate for the insulin results. Insulin induces blood glucose and amino acids to reach cells in the liver, heart, and fat tissues, causing protein production from amino acids, and carbohydrate production of glycogen and triglycerides. Insulin cuts off the usage of fat as a store of energy by inhibiting glucagon secretion. Muscle cells do not eat up carbohydrates in the lack of insulin, so the muscle tends to utilize fat as a store of energy or as gluconeogenesis and deficiency in insulin level regulation results in diabetes mellitus.

Type II is a complicated disorder, and it has many factors that lead to its growth. Among these are the major genetic predisposition and environmental influences, which combine to induce NIDDM. Genetic influences have an important impact on the vulnerability to NIDDM, which is confirmed by research in monozygous twins and other cultural groups [1, 2]. More research has established many genetic variants, which raise the danger of NIDDM[3]. A research carried out by Grant *et al*. [4], reported variants in the TCF7L2 (transcription factor7-like 2) gene in individuals with IDDM in Iceland. A related observation has been repeated in people from various ethnic groups from T2DM. Examples of T2DM-related genes include: IRS1 (insulin receptor substratum 1), INS (insulin), PCSK1 (protein convertase subtilizing / kexin type 1) [3, 5, 6]. The prevalence of T2DM is raised by ecological factors viz, overweight, obesity, extreme body fat, excessive energy consumption and physical inactivity [7]. Gut flora differences and even socioeconomic position may play a major role in the production of IDDM beyond these factors [8].

Diabetes treatment is specifically intended to relieve the effects and reduce micro and macrovascular risks. Oral hypoglycemic medications, viz. biguanide (metformin), thiazolindinediones, sulphanylureas, meglitinides etc., play

a major role in treating NIDDM, but none have been unambiguously effective in preserving euglycaemia and preventing late diabetes complications. Despite many advancements in medicine and a thorough definition of the condition, diabetes appears to be a main cause of mortality and morbidity globally [9]. Plants became the world's main supplier of medicines in the Indian medicine scheme and other ancient societies. Earliest definition of medicinal plant curative properties can be found in Rigveda. About 60 per cent of the world's population uses conventional drugs extracted from herbal plants [10, 11].

Materials and Methods

Plant material preparation

The *Terminalia chebula* fruits were sliced into small pieces and then water cleaned carefully. After washing, the fresh fruits were air dried and then oven dried at 40 °C temperature. The dried fruits then are cut into powder and tanned to produce fine powder. The fruit was dried and eventually collected 250 g of fine powder. The powdered was extracted in a Soxhlet device. Quickly, 100 g plant material dried powdered was Soxhlet removed for 20 hrs with methanol. The concentrate was separated and after that dissolvable was evacuated under decreased weight in rotatory evaporator, to acquire a dark colored powder. The dried concentrate was broken down in typical saline and utilized for research.

Animals

All the investigations were completed in male wistar rats weighing 150 to 200 gm. They were isolate in the animal house in province confines at a surrounding temperature of 24°C approx. and relative mugginess of 40—50% with twelve hours light/dim cycles. All systems utilizing rats were looked into and endorsed by the Animal Ethical Committee that is completely licensed.

Induction of type 2 diabetes mellitus (T2DM)

The T2DM model was built with modification according to process by Reed *et al*. [12]. Rat was prescribed a HFD; (40 percent fat, eighteen percent protein, and forty one percent carbohydrate) ad libitum for the first two week span. Animals were administered intraperitoneally with a small dose of STZ after 2 weeks of nutritional conditioning. The STZ injection die rats with the > 140 mg / dl FBG were deemed diabetic after 6 days, and selected for further tests.

Dose Selection

A maximum of 72 rats were used and specific concentrations of methanol active fractions of *Terminalia chebula* fruit (HFD/*TCF*) (50, 100 and 200 mg / kilogram body wt.) was used to determine the effective dose in treated animals.

Experimental plan

A maximum of 54 rats were used (24 regulars; 30 surviving STZ-diabetic rats). The rats were classified into nine categories, each 6 rats. The TCF fractions were dissolved in vehicle solution (Dimethyl sulfoxide 0.5%; 1ml / kg b.wt) and delivered orally to the respective categories for 45 days using an intragastric tubes. Category 1- Regular rats fed with vehicle only (15% calories as fat); Category 2- Regular rats + *TCF* fraction (200 mg/kg body wt); Category 3- Diabetic rats induced with HFD/STZ treated with vehicle only; Category 4- Diabetic rats induced with HFD/STZ +*TCF* fraction (200 mg/kg body wt.); Category 5- Diabetic rats induced with HFD/STZ + GL (600 µg/kg body wt.)

Biochemical analysis

ELISA kit used rat insulin as normal to assess the serum insulin value whereas HbAlc was evaluated using cation-exchange system [13]. Total and HDL-cholesterol and were assessed by the method of Wybenga *et al.* [14] and serum triglyceride was determined by GPO-PAP, end point assay [15]. Falhot *et al*. [16] technique was used for evaluating FFAs. Blood urea was calculated using the procedure of Diacetylmonoxime (DAM) [17], whereas serum creatinine was estimated using the procedure of alkaline picrate [18] and ALP production was measured using PNPP as a substrate [19]. Finally, LG was calculated by the process of anthrone reagent [20].

Statistical Analysis

Statistical analysis was performed on the data recorded using the SPSS ver. 20.0 software, the data were subjected to ANOVA first between groups and then within groups. All values have been expressed as mean ± Standard mean error.

Results

Determination of effective dose of *Terminalia chebula* fruit

The dose dependent effect is estimated in normal and HFD / STZ-diabetic rats of the *TCF* fraction on plasma glucoses levels. We found elevated levels of plasma glucose relative to normal rats in diabetic rats. The plasma glucose level decreased considerably by 15 days oral administration of the *TCF* fraction (P<0.05) as compared to diabetic rats. Because the *TCF* fraction was effectively fixed as an effective dose at a dose of 200 mg / kg b. wt. (Table 1).

Table 1: Effect of *TCF* on fasting plasma glucose levels in control and HFD/STZ diabetic rats

Parameter	Category 1	Category 2	Category 3	Category 4	Category 5	Category 6
Fasting plasma glucose levels (mg/dL)	95.10 ± 6.1	420.56 ± 11.5##	255.08 ± 8.20	180.21 ± 8.52	115.32 ± 6.1***	109.59 ± 4.3***

Category 1- Regular rats fed with vehicle only (15% calories as fat)
Category 2- Diabetic rats induced with HFD/STZ treated with vehicle only
Category 3- Diabetic rats induced with HFD/STZ + *TCF* fraction (50 mg/kg body wt)
Category 4- Diabetic rats induced with HFD/STZ + *TCF* fraction (100 mg/kg body wt)
Category 5- Diabetic rats induced with HFD/STZ + *TCF* fraction (200 mg/kg body wt)
Category 6- Diabetic rats induced with HFD/STZ + Glibenclamide (600 µg/kg body wt)
Significance of induction of disease is denoted by #
Where # indicates P<0.05, ## indicates P<0.01 and ### indicates P<0.001
Significance of therapeutic level is denoted by *
Where * indicates P<0.05, ** indicates P<0.01 and *** indicates P<0.001

Effect on Serum insulin content, liver glycogen and HbA1c level

Higher levels of liver glycogen and plasma insulin were seen in HFD / STZ controls relative to normal control animals and large increases of HbAlc rates. The content of serum insulin in *TCF* is found higher i.e. 11.82 ± 1.11 IU/L when compared with HFD/STZ-diabetic rats whereas, HbAlc levels were found lower in *TCF* i.e. 0.76 ± 0.18 mg/g of Hb when compared with HFD/STZ-diabetic rats. The liver glycogen was found higher in *TCF* i.e. 6.12 ± 0.65 mg/100gm tissue when compared with HFD/STZ-diabetic rats.

Table 2: Effect of *TCF* on serum insulin content, HbA1c, liver glycogen and renal function marker levels in control and HFD/STZ - diabetic rats

Groups	Category 1	Category 2	Category 3	Category 4	Category 5	Category 6	Category 7	Category 8	Category 9
HbA1c (mg/gm of Hb)	0.62 ± 0.02	0.63 ± 0.03	0.66 ± 0.04	0.64 ± 0.02	2.03 ± 0.41^{x}	0.80 ± 0.32^{y}	0.72 ± 0.22y	0.76 ± 0.18^{y}	0.65 ± 0.09^{xy}
Serum Insulin (IU/L)	14.52 ± 1.35	14.28 ± 1.21	14.69 ± 1.11	14.50 ± 1.23	6.16 ± 1.05^{x}	11.45 ± 1.12^{y}	12.25 ± 1.19y	11.82 ± 1.11^{y}	12.79 ± 1.12^{xy}
Liver glycogen (mg/100gm tissue)	7.45 ± 0.90	7.12 ± 0.84	7.35 ± 0.61	7.22 ± 0.83	4.11 ± 0.52^{x}	5.52 ± 0.83^{y}	6.41 ± 0.72^{y}	6.12 ± 0.65^{y}	7.11 ± 0.32^{xy}
BUN (mg/dL)	20.25 ± 2.12	22.55 ± 3.12	23.91 ± 2.47	23.16 ± 2.20	33.52 ± 0.31^{x}	29.75 ± 0.32^{y}	26.23 ± 0.53^{y}	27.59 ± 0.52^{y}	25.96 ± 0.22^{xy}
Creatinine (mg/dL)	3.21 ± 0.41	3.18 ± 0.45	3.02 ± 1.32	3.11 ± 0.51	4.29 ± 0.12^{x}	3.62 ± 0.40^{y}	3.78 ± 0.32^{y}	3.68 ± 0.51^{y}	3.89 ± 0.65^{xy}
Albumin (µg/d)	139.05 ± 1.35	135.15 ± 3.70	130.82 ± 3.11	133.11 ± 2.15	225.72 ± 2.75^{x}	196.52 ± 3.81^{y}	183.15 ± 2.18^{y}	177.65 ± 1.32^{y}	165.72 ± 2.60^{xy}

Each value is mean ± SD for 6 rats in each category
x: $p<0.05$ by comparison with normal rats
y: $p<0.05$ by comparison with HFD/STZ- diabetic rats
-: No significance.

Table 3: Effect *TCF* on serum total cholesterol, HDL, LDL, VLDL, triglycerides and free fatty acid levels in control and HFD/STZ - diabetic rats.

Groups	Category 1	Category 2	Category 3	Category 4	Category 5	Category 6	Category 7	Category 8	Category 9
Total cholesterol (mg/dL)	95.65 ± 1.89	101.57 ± 1.36	99.91 ± 1.13	100.24 ± 3.79	214.03 ± 2.58^{x}	135.83 ± 1.22^{y}	104.09 ± 1.15^{y}	113.18 ± 1.19^{y}	114.29 ± 1.38^{xy}
Triglyceride (mg/dL)	13.39 ± 1.21	14.47 ± 1.19	13.88 ± 1.01	14.24 ± 1.25	41.70 ± 2.54^{x}	20.57 ± 2.47^{y}	18.31 ± 2.04^{y}	19.26 ± 2.21^{y}	17.19 ± 2.72^{xy}
Free fatty acids (mg/dL)	79.20 ± 2.12	84.66 ± 3.31	83.02 ± 3.16	83.73 ± 2.52	144.85 ± 2.49^{x}	117.52 ± 2.13^{y}	95.43 ± 2.31^{y}	101.52 ± 2.03^{y}	91.49 ± 2.11^{xy}
HDL Cholesterol (mg/dL)	53.69 ± 2.30	53.85 ± 2.45	54.47 ± 2.32	54.12 ± 2.01	29.52 ± 1.50^{x}	40.33 ± 2.13^{y}	42.77 ± 1.06^{y}	42.03 ± 1.29^{y}	48.45 ± 2.71^{xy}
LDL Cholesterol (mg/dL)	33.70 ± 1.32	37.05 ± 1.51	42.75 ± 0.91	40.29 ± 1.29	54.38 ± 2.01^{x}	40.32 ± 2.32^{y}	37.85 ± 3.54^{y}	38.89 ± 2.23^{y}	36.65 ± 2.23^{xy}
VLDL Cholesterol (mg/dL)	14.45 ± 1.85	16.29 ± 1.73	16.75 ± 1.61	16.41 ± 1.52	32.32 ± 2.48^{x}	21.40 ± 2.74^{y}	19.10 ± 2.42^{y}	20.51 ± 2.01^{y}	17.44 ± 2.61xy

Each value is mean ± SD for six rats in each category
x: $p<0.05$ by comparison with normal rats.
y: $p<0.05$ by comparison with HFD/STZ- diabetic rats.
-: No significance.

Effect on Total cholesterol, HDL, LDL, VLDL, triglycerides and free fatty acid levels

The HFD/STZ induced group showed significant elevation of the level of total cholesterol (95.65 ± 1.89 mg per dL to 214.03 ± 2.58 mg per dL), triglycerides (13.39 ± 1.21 mg per dL to 41.70 ± 2.54 mg per dL), free fatty acids (79.20 ± 2.12 mg per dL to 144.85 ± 2.49 mg per dL), LDL (33.70 ± 1.32 mg per dL to 54.38 ± 2.01 mg per dL), VLDL (14.45 ± 1.85 mg per dL to 32.32 ± 2.48 mg per dL) respectively and decreased the level of HDL (53.69 ± 2.30 mg per dL to 29.52 ± 1.50 mg per dL). Diabetic disease was prevented from treatment with *TCF* and glibenclamide/HFD at dose level and serum cholesterol, TG, LDL, FFA, VLDL were reduced significantly and HDL levels increased significantly.

Effect on Renal function markers in serum

TCF have been tested to determine renal function in serum in HFD / STZ group on renal markers (BUN, Scr and ALP). In the HFD / STZ group the control group has substantially improved ($p<0.05$) compared with control category. The addition of *TCF* and GL has substantially decreased in these markers ($P<0.05$) relative to the category HFD/STZ.

Discussion

In this study, the ability of the *TCF* extract to reduce increased blood sugar to regular glycemic level is an important liver trigger to return during experimental diabetes to its normal homeostasis. Possible mechanism by which *TCF* produce its hypoglycemic action in diabetic rats can potentiate the effect of serum insulin by elevating the insulin pancreatic secretion from the existing p-cells. There are similar reports to show that medicinal plants with hypoglycemic properties can affect the circulation of insulin levels [21, 22].

DM is related to a pronounced decline in liver glycogen levels [23]. The reduced glycogen store was due to decline in activity of glycogen synthase and an increase in glycogen

phosphorylase activity. Oral administration of *TCF* fractions [21] and significantly increases levels of hepatic glycogen in STZ or HFD / STZ-diabetic rats, possibly due to glycogen synthase system reactivation, as a result of elevated secretion of insulin. HbA1c was shown to be growing in diabetes over a longer period. Therefore, a highly sensitive index of glycemic control should be measured for HbA1c. In diabetic animals, glycosylated hemoglobin increased significantly, and this rise was found to be directly proportional to the amount of fasting blood glucose. Oral administration of active *TCF* fractions [21, 24] prevents a significant increase in diabetic rat glycosylated hemoglobin levels. This may be due to improved glycemic control of fractions of plants.

The abnormal high serum lipid concentration in diabetic subjects is basically due to the free fatty acids increased mobilization from fat depots as insulin is needed to inhibit hormone-sensitive lipase. It is well known that there will be a rise in VLDL, LDL, TC and triglyceride with a decline in HDL cholesterol contributing to the secondary complications in uncontrolled type 2 DM [25]**.** High serum / tissue levels such as cholesterol, triglycerides and free fatty acid have been reported in diabetic rats. HDL carries cholesterol into the liver from peripheral tissues and it functions against coronary heart disease as a preventive factor. There are several findings that HDL plasma cholesterol is small in insulin-deficient diabetics untreated [26, 27], consistent with the decrease in HDL turnover. The surface apoproteins and lipids of HDL are found to contribute during hydrolysis with LPL, VLDL and chylomicrons. The glycosylation of apoprotein B lysyl residue and decreasing the affinity with the LDL receptor and thus decreasing the metabolism may lead to increased LDL-cholesterol [28]. LDL-cholesterol was found to be especially susceptible to atherogenesis in diabetic patients to form lipid peroxides [29]**.** While, the levels of free fatty acid, cholesterol and triglycerides are elevated in STZ or HFD/STZ diabetic rats. Oral administration of *TCF* [30, 31, 32] fractions standardized these effects, controlling hydrolysis and selective absorption by various tissues of certain lipoproteins and their metabolism.

Type 2 diabetic animals infected with STZ reported a substantial rise ($p<0.05$) in BUN and creatinine relative to normal group animals. Hyperglycemia can lead to an increase in serum urea and creatinine levels that causes osmotic diuresis and extracellular fluid volume depletion. Several studies have also found increased correlation between serum urea and creatinine in patients with diabetes for TCF [33, 34, 35].

ALP is omnipresent in nature; its primary function is to provide inorganic phosphate for cell growth by using hydrolysing external phosphate esters which fail to penetrate the cytoplasmic membrane [36], and also ALP is the prototype of these enzymes that reflect pathological changes in biliary flow. Oral administration of TCF [37, 38] extracts to lower the elevated serum enzyme level. Rise in albumin levels were noticed in STZ-control rats when compared to normal control animals. Oral treatment with TCF [39] fractions and Glibenclamide had shown the significant recovery in albumin levels.

Conclusion

In conclusion, this research demonstrates the promising anti-hyperglycemic effects of phytoconstituents in STZ-induced Diabetic Rats on *Terminalia chebula* fruit. It was inferred from the above discussion that methanol plant extracts of TCF at dose (200 mg / kg) exhibited substantial antihyperglycemic behaviour in diabetic rats induced by STZ. This extract also demonstrated change in the parameters such that it could be of use in the treatment of diabetes. Therefore the production of anti-diabetes lead molecles may be tested for these anti-hyperglycemic agents and further research to determine mechanics of action may lead to improved diabetes care and regulation. The research can indicate that this extract has a hypoglycemic effect of synergetic, demonstrated by increased serum insulin levels, reduced serum lipid levels and thus plant extracts therapeutic benefit in rats to battle diabetic disease. Further study is therefore required to understand the exact cause of hypoglycemic effects.

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