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# *Antidiabetic effect of chebulic acid in streptozotocin induced diabetic rats*

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## ABSTRACT

**Ethanopharmacological relevance:** Terminalia chebula is widely incorporated to treat diabetes and its complications; chebulic acid was used to prevent advanced glycation end products-induced endothelial cell dysfunction, however the antidiabetic effect of chebulic acid is questionable.

**Aim of study:** The present study was designed to investigate the effect of chebulic acid in streptozotocin induced diabetes in rats.

Materials and methods: Chebulic acid (CA) was isolated from T. chebula. $LD_{50}$ and acute toxicity studies of CA was done. Chebulic acid at doses of 25 and 50 mg/kg was administered to diabetic rats (STZ; 50 mg/kg i.p.). CA and glibenclamide (10 mg/kg) were administered for 28 days and various biochemical parameters were monitored in order to analyze antidiabetic effect.

**Results:** $LD_{50}$ was found to be 251 mg/kg; 25 and 50 mg/kg dose were selected as no toxic symptoms were observed at both doses, except slight diarrhea. CA at both doses significantly increased glucose and sucrose tolerance. The statistical data indicated that CA significantly ($p<0.001$) reduced blood glucose level, glycosylated hemoglobulin, fructosamine, glucose 6 phosphate dehydrogenase, glucose 6 phosphatase, lactate dehydrogenase, glycogen phosphorylase, fructose 1,6 bisphosphatase, and urine glucose excretion. CA significantly decreased glycoprotein levels where as glycogen level, serum insulin, glycogen synthetase, and hexokinase were significantly increased.

**Conclusion:** CA significantly regulated hyperglycemia and could be a therapeutic agent for regulating several pharmacological targets for treatment or prevention of diabetes.

**Keywords**: Antidiabetic; Chebulic acid; Glibenclamide; $LD_{50;}$ Streptozotocin

## 1. INTRODUCTION

Diabetes mellitus is not a single disease entity but rather a group of metabolic disorders characterized by defective regulation of carbohydrate, fat, and protein or in other words it is a metabolic disorder characterized by hyperglycemia, glycosuria, hyperlipidemia, negative nitrogen balance and sometime ketonemia. The incidence of Diabetes mellitus is on the rise worldwide (Aslan et al., 2006).

Researchers have no doubt that nature is still the preeminent synthetic chemist and that in plants particularly, there is almost infinite reserves of fascinating chemical constituents with actual and potential effects on human body (Edwin and Sheeja, 2006). The World Health Organisation (WHO) has also recommended the evaluation of the plants effectiveness in conditions where safety is point of concern (Day, 1998). This has leaded an increasing demand of research on antidiabetic natural products which produces minimal or no side effects (Rao et al., 1999).

*Terminalia chebula* Retz. (Combretaceae) is native to India and Southeast Asia and has been used traditionally to treat various diseases. In modern era it has been used as antidiabetic (Senthilkumar et al., 2006), cytoprotective (Saleem et al., 2002), renoprotective (Rao and Nammi, 2006), antioxidant (Naik et al., 2004) and cardio protective (Suchalatha and Devi, 2005). Tannins isolated from *T. chebula* are cytoprotective (Lee et al., 1995), antioxidant (Klika et al., 2004), and α-glucosidase inhibitor (Gao et al., 2007). Although antidiabetic effect of *T chebula* has been verified and chebulic acid (CA) was used to prevent advanced glycation end products-induced endothelial cell dysfunction (Lee et al., 2010), effect of CA on diabetes has not been published. Hence the present study was carried out to evaluate the antidiabetic activity of CA.

## 2. MATERIALS AND METHODS

### *2.1. Chemicals*

All the chemicals were of analytical grades (Loba Chem, Mumbai); streptozotocin (STZ) was procured from Sigma Aldrich, Mumbai; glibenclamide from Torrent Pharmaceutical Pvt. Ltd., Mumbai; glucometer and strips from Dr. Morepen, New Delhi; diagnostic kits from Lab Care Diagnostic (India) Pvt. Ltd.

### *2.2. Plant material*

Dried fruits of *T. chebula* were purchased from local market, Mandsaur, M.P.; India; identified by Prof. Gyanendra Tiwari, Scientist, Government College of Horticulture, Mandsaur (M.P.). A voucher specimen (BRNCP/TC/009/2009) was deposited in the herbarium of Department of Pharmacognosy, B. R. Nahata College of Pharmacy, Mandsaur (M.P.).

### *2.3. Extraction and isolation*

Fruits of *T. chebula* (5 kg) were coarsely powdered; defatted with petroleum ether (60°C-80°C); dried and filled in soxhlet apparatus for extraction with absolute ethanol as solvent. The extraction was carried out for a period of 72 hrs. The extract obtained was dried in vacuum to remove excess solvent. Ethanolic extract obtained was resuspended in $H_2O$, and then extracted successively with n-hexane, chloroform, ethyl acetate (EtOAc), and n-butanol. Extract obtained after extraction with ethyl acetate was dried. Dried compound was subjected to column chromatography (silica gel) and eluted by methanol in ethyl acetate (30:70, v/v). The obtained fraction was further purified by Sephadex LH-20 column chromatography using methanol as the eluent. RP-HPLC (Waters, USA) with C-18 column (5µm, 250 x 4 mm, Lichro Cart, Merck, Germany) was used for further separation and estimation; formic acid and acetonitrite were used as eluent and quantification of compound was done using calibration curve of standard compound. Data of FTIR, $^1$H NMR and MS spectroscopy of Lee *et al.*, were used to identify the compound (Lee et al., 2007).

### *2.4. Experimental animals*

Healthy adult male albino wistar rats (200-250 g), in-house bred at the Animal House of B.R.N.C.P. Mandsaur, M.P. India were used for the study. Rats were housed in polypropylene cages lined with husk in standard environmental conditions (temperature 25 ± 2°C, relative humidity 55 ± 10% and 12:12 light:dark cycle). The rats were fed on a standard pellet diet (Pranov Agro Pvt Ltd, Vadodara, Gujrat, India) *ad libitum* and had free access to water. The experiments were performed after approval of the protocol by the Institutional Animal Ethics Committee (IAEC reg. no. 918/AC/05/CPCSEA) and were carried out in accordance with the current guidelines for the care of laboratory animals.

### 2.5. *Acute oral toxicity study*

Miller and Tainter (graphical) method was used to calculate $LD_{50}$. The probit values were plotted against log dose and then dose corresponding to probit 5 was calculated. $1/5^{th}$ and $1/10^{th}$ of $LD_{50}$ was selected as dose for further study (Ghosh, 2008).

The rats were randomly divided into three equal groups (n = 6/group); Group I served as control and received distilled water; Group II received single oral dose of CA (25 mg/kg) and Group III received single oral dose of 50 mg/kg of CA. During the treatment, each animal was observed daily for overt signs of toxicity (salivation, lachrymation, squinted eyes, writhing, convulsions, tremors, yellowing of fur, loss of hair), stress (erection of fur and exophthalmia), behavioral

abnormalities (impairment of spontaneous movement, climbing, cleaning of face and ataxia and other postural changes) and aversive behavior (biting and scratching behavior, licking of tail, paw and penis, intense grooming behavior and vocalization) and diarrhea. The animals were observed for gross behavioral, neurological, autonomic and toxic effects at short intervals of time for 24 h and then daily for 14 days. Food consumption was monitored daily and body weights were recorded weekly. On 14th day, animals were sacrificed and all the organs were removed for gross pathological examination (Irwin, 1968).

*2.6. Experimental design*

Antidiabetic activity of CA was assessed in glucose loaded hyperglycemic and streptozotocin-induced diabetic rats. In all studies, the animals were fasted overnight for 16 h with free access to water throughout the duration of the experiment.

*2.6.1. Oral glucose tolerance test*

Healthy rats were divided into four groups of six animals each: Group I served as control received only vehicle (distilled water), group II served as standard received glibenclamide (10 mg/kg), group III and IV received CA orally at the dose level of 25 mg/kg and 50 mg/kg respectively. All the animals were given glucose (2 g/kg) 60 min after dosing. Blood samples were collected from the retro-orbital plexus of the eye just prior to (0 h) and at 30, 60, 90 and 120 min after the glucose loading and blood glucose levels were estimated (Prakasam et al., 2003).

*2.6.2. Assessment of α-glucosidase activity*

The rats were fasted for 16 h, randomly divided into four groups (n = 6/group) and pre-treatment blood glucose levels obtained. They were orally administered either with water or CA or glibenclamide (n = 6/group) as described above. One hour later, all these rats were orally loaded with 5ml/kg bw of 40% (w/v) sucrose solution. Blood samples were collected from the tails of sucrose loaded rats at hourly intervals for 4 h. Serum was separated (at 27°C) and glucose concentration determined for the assessment of *α*-glucosidase activity in terms of oral sucrose tolerance in rats (Ratnasooriya et al., 2004).

*2.6.3. Evaluation of CA in streptozotocin-induced diabetic rats*

Experimental diabetes was induced by single intraperitoneal injection of 55 mg/kg of STZ, freshly dissolved in cold citrate buffer, pH 4.5. Following the STZ injection, rats were given drinking water supplemented with sucrose (15 gm/L) for 48 h, to limit early mortality as stores of insulin are released from damaged pancreatic islets. Control animals received only citrate buffer. After 5 days of STZ injection, animals with fasting blood glucose above 250 mg/dl were considered as diabetic and included in the study. Diabetic rats should be given daily subcutaneous injections of long-acting insulin (2–4 U/rat, Human Mixtard, Abbott India Ltd., Mumbai, India) to maintain blood glucose levels in a desirable range (Nagle et al., 2006).

The animals were randomly assigned into five groups of six animals each and received the following treatments: group I (normal control) received vehicle (water only), group II (diabetic control) also received vehicle, group III received glibenclamide (10 mg/kg) where as doses of 25 and 50 mg/kg of CA was given orally to group IV and V respectively.

The freshly prepared solutions were orally administered daily for 28 days. Body weights and blood glucose analysis was done weekly on overnight fasted animals. At the end of the experimental period, the animals were fasted overnight and blood was collected for various biochemical estimations. The animals were sacrificed by cervical decapitation.

*2.6.4. Biochemical analysis*

Urinary glucose (UG), glycosylated hemoglobulin (GHb), lactate dehydrogenase (LDH) and glucose 6 phosphate dehydrogenase (G6PDH) were assessed using commercially available diagnostic kits. Blood glucose level (BG) was assessed using glucose strips (Dr. Morphen's glucostrip) and Sahu method was used for estimation of fructosamine (Fs) (Sahu and Sarkar, 2008), Serum insulin was analyzed by commercial pathology laboratory (Choksi Laboratories Limited; Indore; M.P.).

Liver were dissected, washed with ice cold saline and homogenized in 0.1 M Tris HCl buffer, pH 7.4. The supernatant was used for the assay of enzyme activity. Hexokinase was assayed by Brandstrup method (Brandstrup et al., 1957). Koide method was adopted for glucose 6 phosphatase estimation (Koide and Oda, 1959) and Gancedo method for frustose 1,6 bisphosphate estimation (Gancedo and Gancedo, 1971). Glycogen was assessed by method of Maiti (Maiti et al., 2004). The activities of glycogen synthetase and glycogen phosphoralase were assayed by the method of Lelior, and Cornblath respectively (Lelior and Goldenberg, 1962; Cornblath et al., 1963).

Another portion of liver tissue was used to assay glycoproteins like hexoses, hexosamine, fucose and sialic acid. The tissue were defatted and then suspended in 3.0 ml 2M HCl and heated at 90 °C for 4h; cooled and neutralized with 3.0 ml 2M NaOH. Samples were used for the estimation of glucoproteins; Niebes method was used to estimate hexose (Niebes, 1972), hexosamine was estimated by Wagner method (Wagner, 1979). Fucose and sialic were estimated by

the method of Dische and Warren method respectively (Dische and Shettles, 1948; Warren, 1959).

*2.6.5. Statistical analysis*

All values are expressed as mean ± S.E.M. Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Dunnett's tests. The results were considered statistically significant if $P < 0.05$.

## 3. RESULTS

*3.1. Isolation and characterization*

The compound was isolated from *T. chebula* as yellow powder with a % yield of 0.07% w/w; % purity of 97.04% w/w and melting point of 195-208°C. The IR spectrum showed the main peaks at 3560-3500, 3000-3100, 1600, 725-680 (data not shown). $^1$H NMR data showed (400 MHz, Deuterium Oxide) δ 6.75 (s, 1H), 5.50-5.41 (m, 2H), 3.98 (t, *J*=5.1 Hz, 1H), 3.38 (q, *J*=5.0 Hz, 1H), 3.06 (dd, *J*=12.5, 5.1 Hz, 1H), 2.73 (dd, *J*=12.5, 5.1 Hz, 1H) and from above obtained data compound was identified as chebulic acid.

*3.2. $LD_{50}$ and acute toxicity study*

Experiment was carried out on normal healthy rats. $LD_{50}$ of CA was found to be 251 mg/kg. In acute toxicity, CA at 25 mg/kg and 50 mg/kg did not show any behavioral and neurological changes. Mild diarrhea was observed and no gross pathological changes were seen at both doses; thus 25 mg/kg and 50 mg/kg dose were selected for future study.

*3.3. Effect of CA on glucose tolerance in normal and diabetic rats*

The results obtained for the oral glucose tolerance test in normal and diabetic rats are shown in Table 1. As shown, glucose challenge in normal and diabetic rats elevated the blood glucose levels with in 30 min; glibenclamide (10 mg/kg) maintained blood glucose level in normal range through out the study where as elevation in blood glucose level was observed in CA (25 mg/kg and 50 mg/kg) treated group within 30 min and with progression of time (60 min-120 min) it maintained the glucose level in normal range. The glucose tolerance by glibenclamide and CA treated normal rats was almost same.

A slight elevation in blood glucose level of diabetic rats was observed in glibenclamide treated rats (137.78±3.89) which was controlled with in 60 min and maintained through out study period. CA at 25 mg/kg showed elevation in blood glucose level upto 90 min and took 2 h to check glucose level where as CA at 50 mg/kg brought blood glucose level in normal range within 90 min of glucose challenge. CA showed a dose dependent effect in glucose tolerance.

*3.4. Effect of CA on α-glucosidase activity (Sucrose challenge)*

Control group show elevation in blood glucose level, showed in Table 1. Glibenclamide and CA (at both doses) showed almost similar pattern of reduction of blood glucose level. Oral sucrose challenge showed that glibenclamide and CA equally inhibit α-glucosidase.

| Groups | Pretreatment levels | Post treatment levels | | | |
|---|---|---|---|---|---|
| | | 30 min | 60 min | 90 min | 120 min |
| **Normoglycemic** | | | | | |
| NC | 85.67 ± 1.45 | 135.30 ± 4.80 | 116.71 ± 4.67 | 98.37 ± 2.96 | 92.67 ± 1.45 |
| SG | 90.33 ± 2.33 | 105.33 ± 1.20$^{**£}$ | 86.00 ± 1.53$^{**}$ | 83.67 ± 1.45$^{**}$ | 81.67 ± 0.88$^{**}$ |
| CA 25 | 89.67 ± 1.76 | 131.30 ± 2.40$^{*££}$ | 106.70 ± 2.03$^{*£}$ | 94.67 ± 1.20$^{*£}$ | 83.67 ± 1.42$^{**}$ |
| CA 50 | 88.86 ± 2.48 | 133.33 ± 1.67$^{££}$ | 102.76 ± 1.45$^{*£}$ | 89.29 ± 1.54$^{**}$ | 80.48 ± 1.04$^{**}$ |
| **Diabetic** | | | | | |
| NC | 360.84 ± 21.50 | 420.54±23.87 | 438.64±19.70 | 470.22±17.62 | 408.42±20.04 |
| SG | 350.33±6.21 | 237.78±3.89$^{***£}$ | 116.67±8.85$^{***}$ | 108.52± 7.67$^{***}$ | 107.74±5.13$^{***}$ |
| CA 25 | 366.12±3.45 | 267.19±14.26$^{***£££}$ | 162.58±1.73$^{***£££}$ | 130.25±6.23$^{***££}$ | 112.64±4.06$^{***}$ |
| CA 50 | 348.26 ± 3.85 | 258.67 ± 8.24$^{***£££}$ | 150.33 ± 4.35$^{***£££}$ | 120.86 ± 6.78$^{***£}$ | 98.48 ± 4.54$^{***}$ |
| **Glucosidase inhibition (sucrose tolerance)** | | | | | |
| NC | 83.6 ± 2.64 | 130.5 ± 2.23 | 118.4 ± 3.24 | 106.2 ± 2.46 | 93.4 ± 3.62 |
| SG | 88.5 ± 4.32 | 96.5 ± 4.28$^{**£}$ | 85.4 ± 2.58$^{**}$ | 80.6 ± 2.28$^{**}$ | 76.5 ± 2.45$^{**}$ |
| CA 25 | 83.4 ± 2.35 | 99.3 ± 2.86$^{**£}$ | 88.3 ± 1.59$^{**}$ | 85.4 ± 3.54$^{**}$ | 83.5 ± 4.87$^{**}$ |
| CA 50 | 90.5 ± 6.45 | 105.6 ± 1.54$^{**£}$ | 93.5 ± 2.57$^{**}$ | 87.6 ± 2.87$^{**}$ | 83.5 ± 2.37$^{**}$ |

*NC: normal control (saline), SG: standard glibenclamide (10 mg/kg), CA 25: chebulic acid (25 mg/kg), CA 50: chebulic acid (50 mg/kg); N=6; Values are expressed as mean ± SEM, where $^{*}$ p < 0.05, $^{**}$ p < 0.01, $^{***}$p < 0.001 vs normal control and $^{£}$ p < 0.05, $^{££}$ p < 0.01, $^{£££}$p < 0.001 vs pretreatment group.*

***Table 1 :*** **Effect of chebulic acid (CA) on glucose tolerance (normoglycemic and hyperglycemic rats) and sucrose tolerance in normoglycemic rats**

*3.5. Effect of CA on blood glucose level of diabetic rats*

The effect of repeated oral administration of CA on blood glucose levels in STZ-diabetic rats is presented in table 2. CA at both doses caused significant ($p<0.001$) reduction of blood glucose levels which was related to dose and duration of treatment. CA 50 mg/kg showed maximum decrease (79.11%) in glucose level which was comparable to glibenclamide (78.19%).

| Groups | BGL (mg/dl) | GHb (% $HbA_1C$) | Fs (µmol/mg protein) | G6PHD (g/dl) | SI (µU/ml) | UG (mg/24 h) |
|---|---|---|---|---|---|---|
| NC | 111.8±2.78 | 5.17±0.60 | 198.36 ± 8.28 | 5.562±0.39 | 14.50±0.76 | 0.0±0.0 |
| DC | 500.8±19.70£££ | 12.83±0.70£££ | 318.57±23.54£££ | 14.48±0.23£££ | 5.83±0.79£££ | 3459±76.42£££ |
| DS | 109.2±5.13*** | 6.32 ±0.58*** | 204.96±4.59*** | 6.42± 0.26*** | 8.28 ±0.45*** | 854 ±45.65*** |
| DC 25 | 118.2±3.91*** | 5.67±0.33*** | 208.56±9.24*** | 6.26±0.29*** | 11.67±0.17 *** | 988±55.15*** |
| DC 50 | 104.6 ± 8.58*** | 5.59 ± 0.46*** | 200.46 ± 6.57*** | 6.24 ± 0.18*** | 13.45 ± 0.24*** | 896 ± 42.52*** |

NC: normal control (saline), DC: diabetic control (saline), DS: standard drug (glibenclamide 10mg/kg), DC 25: diabetic + chebulic acid (25 mg/kg), DC 50: diabetic + chebulic acid (50 mg/kg); N=6; Values are expressed as mean ± SEM, where ***p < 0.001 vs diabetic control; £££p < 0.001 vs normal control.

**Table 2: Effect of different dose of CA on blood glucose level (BGL), glycosylated hemoglobulin (GHb), fructosamine (Fs), glucose 6 phosphate dehydogenase (G6PHD), insulin (SI) and urine glucose excretion (UG)**

*3.6. Effect of CA on biochemical alterations in STZ-induced diabetic rats*

After 28 days treatment period, it was observed that the animals treated with CA at both doses (25 and 50 mg/kg) caused significant (p<0.001) decrease in GHb, Fs and G6PHD where as insulin level was significantly (p<0.001) increased at the end of the study when compared to diabetic control group (Table 2).

The urine sugar in treated diabetic groups decreased by 71.43% and 74.09% by CA at 25 and 50 mg/kg dose respectively; the decrease was comparable with glibenclamide (75.31%). However, with none of treatment, the values were restored to normal.

STZ caused a significant (p<0.001) decrease in glycogen, glycogen synthetase and hexokinase as compared to normal animals. Administration of CA at both doses caused significant (p<0.001) increase in levels which were comparable to glibenclamide.

The changes in activity of glycogen phosphorylase, G6P, F 1,6 P and LDH of normal, diabetic control and experimental groups are shown in Table 3. The activities of hepatic gluconeogenic enzymes were significantly increased in diabetic rats. Treatment by CA and glibenclamide significantly reversed the changes in activities of hepatic enzymes. CA at both doses (25 and 50 mg/kg) showed more prominent effect when compared to glibenclamide.

Table 3 presents the changes in hepatic glycoproteins (hexoses, hexosamine, fucose and sialic acid). Significantly higher levels of glycoproteins were observed in liver of diabetic control rats when compared to normal rats. CA showed a dose related significant (p<0.001) reduction in glycoproteins.

| Parameters | NC | DC | DS | DC 25 | DC 50 |
|---|---|---|---|---|---|
| Glycogen (mg/gm wet tissue) | 58.67±2.20 | 25.50±1.19£££ | 46.35 1.25*** | 47.17±0.60*** | 50.38 ± 1.05*** |
| Glycogen synthetase (µm of uridine diphosphate formed/h/mg protein) | 890.54 ±56.42 | 395.82± 42.95£££ | 630.56 ±54.38*** | 720.92 ±34.60*** | 775.84 ± 30.84*** |
| Glycogen phosphorylase (µm of phosphate liberated/h/mg protein) | 586.19 ±34.64 | 782.45 ±32.04£££ | 686.67 ±28.45*** | 624.48 ±20.54*** | 600.87±30.24*** |
| Hexokinase (µm of glucose 6 phosphate formed/h/mg protein) | 269.3±7.29 | 148.8±6.99£££ | 198.2 ±3.48*** | 252.5±4.01*** | 260.4±2.54*** |
| G 6 P (µm of phosphate liberated/h/ mg protein) | 9.5±0.43 | 42.67±2.39£££ | 18.33± 2.67*** | 13.00±0.52*** | 11.08 ±0.68*** |
| F 1,6 P (µm of phosphate liberated/h/ mg protein) | 437.56 ±45.33 | 830.97± 24.87£££ | 548.28 ±36.58*** | 512.28 ±34.56*** | 483.40±20.24*** |
| LDH (µm of pyruvate formed/h/mg) | 230.7±4.15 | 360.7±9.20£££ | 300.8 ±5.64** | 261.3±8.11*** | 250.9±12.46*** |
| Hexoses (mg/g) | 36.42 ±3.40 | 60.45± 2.96£££ | 42.35 ±4.56*** | 37.84 ±2.56*** | 37.12±5.32*** |
| Hexosamine (mg/g) | 15.28 ±3.54 | 31.64 ±3.24£££ | 18.54 ±1.72*** | 16.37± 2.56*** | 16.00±3.85*** |
| Fucose (mg/g) | 12.84 ±2.98 | 23.15± 1.91£££ | 16.57± 1.75*** | 14.27 ±2.35*** | 13.87±3.28*** |
| Sialic acid (mg/g) | 9.54 ±4.52 | 16.21± 3.24£££ | 11.24 ±2.34*** | 10.83± 3.27*** | 10.42±2.67*** |

NC: normal control (saline), DC: diabetic control (saline), DS: standard drug (glibenclamide 10mg/kg), DC 25: diabetic + chebulic acid (25 mg/kg), DC 50: diabetic + chebulic acid (50 mg/kg); N=6; Values are expressed as mean ± SEM, where ***p < 0.001 vs diabetic control, **p < 0.01 vs diabetic control and £££p < 0.001 vs normal control.

**Table 3: Effect of CA on biochemical parameters**

## 4. DISCUSSION AND CONCLUSION

The present manuscript discusses about the antidiabetic effect of chebulic acid isolated from *T. chebula* on streptozotocin induced diabetic rats. $LD_{50}$ of

CA was found to be 251 mg/kg. Acute toxicity studies at 25 and 50 mg/kg doses of CA revealed the non-toxic effect of CA. There was no lethality or any toxic reactions found with the selected dose until the end of study period.

Streptozotocin (STZ) is the most prominent diabetogenic chemical used in diabetes research as it resembles many features of human diabetes mellitus i.e. high blood glucose level, upto 93 % decrease in insulin level, high glucagon level, abnormal glucose tolerance and development of physiological effect (Arulmozhi et al., 2004).

The GTT studies in normal rat revealed a maximum fall of 23.30% in 1 h by CA at 50 mg/kg where as in diabetic animals CA brought glucose level in normal range after 90 min. CA inhibited rise of blood glucose level following oral sucrose challenge test within 30 min, provides evidence for inhibition of α glucosidase activity. From results it may be said that CA reduce postprandial hyperglycemia and maintain blood glucose level even it is challenged by glucose or sucrose overload. Results of the study have also shown that CA attenuated physiological condition like cachexia, polydipsia, polyuria and polyphagia.

Previously many mechanisms have been established for antihyperglycemic activity via converting glucose to glycogen by glycogen synthetase and promotion of glycogen storage in liver (Jayakody and Ratnasooriya, 2008); retardation of absorption of glucose through the inhibition of carbohydrate-hydrolyzing enzyme α glucosidase (Bhandari et al., 2008); enhancement of insulin activity (Anderson and Polansky, 2002); enhanced glucose uptake and metabolism and insulinomimetic activity (Kadowaki, 2000). Previous researchers also postulate that plants or active metabolites act by several mechanisms at a time.

*T. chebula* have been used as antihyperglycemic agent and stimulated insulin secretion from remnant β cells or from regeneration of β cells. Oral administration of CA for 28 days caused a significant decrease in blood glucose level and significantly increased the insulin level comparable to that of *T. chebula* (Senthilkumar et al., 2006), further clarified the antidiabetic and insulinogenic activity of CA.

Hyperglycemia also leads to nonenzymatic glycation, resulting structural and functional changes in soluble and insoluble protein molecules – like hemoglobin in blood. Uncontrolled GHb augments development and progression of microvasular and macrovascular complications. Strict control of glucose causes a subsequent reduction in GHb level (Krishnamurti & Steffes, 2001). CA showed a prominent effect on reduction of glycosylate hemoglobulin, which further confirms antidiabetogenic effect, standard glibenclamide also showed the same results. It is interesting that CA brought down the elevated level of fructosamine in diabetic rats to nearly normal level after treatment for 28 days, effect was comparable with standard glibenclamide. CA significantly reduced urine glucose excretion but neither CA nor glibenclamide were able to abolish glucose excretion completely.

Conversion of glucose to glycogen in liver is dependent on extracellular glucose concentration and on availability of insulin. CA significantly restored level of hepatic glycogen, which further confirms insulinogenic effect of CA and regulation of glycogenesis by CA. Glycogen is regulated by enzyme glycogen synthase and glycogen phosphorylase. The reduction in the glycogen store in diabetes is mainly due to reduction in activity of glycogen synthase and increased level of glycogen phosphorylase (Senthilkumar and Subramanian, 2008). CA restored level of hepatic enzymes; may be due to increased insulin level attributing to regulation of glycogenesis i.e. by decreasing glycogen phosphorylase and increasing glycogen synthase.

Hexokinase act as initiator of glucose metabolism as it causes phosphorylation of glucose. In diabetes a reduced level of hexokinase was observed resulting in diminished glucose consumption and elevated blood glucose level. STZ caused reduction in hexokinase synthesis by decreasing RNA coding for hexokinase enzyme; lack of insulin also causes inactivation of hexokinase (Nehal and Baquer, 1989). In present study, a promising release of hexokinase was seen in CA; which may be due to increased insulin secretion, further supporting insulinogenc effect of CA.

STZ elevated LDH concentration (responsible for converting lactic acid into pyruvic acid) and disturb $NAD^+$/NADH ratio resulting in regulation of redox pathways. CA caused a reduction in LDH enzyme may be due to regulation of $NAD^+$/NADH ratio by oxidation of NADH. In diabetes an elevation in hepatic glyconeogenic enzyme level was observed due to activation or increased synthesis of enzymes contributing to the increased glucose production during diabetes or inhibition of glycolysis and gluconeogenesis or regulation of cAMP (Senthilkumar and Subramanian, 2008). CA showed significant reduction in hepatic glyconeogenic enzymes (glucose 6 phosphotase and fructose 1,6 bisphosphates) and glucose 6 phosphate dehydrogenase further support its antidiabetic effect.

Increase in glycoprotein components has been associated with severity and duration of diabetes (Rahman et al., 1990). Diabetes and deficiency of insulin causes derangement of glycoproteins (hexoses, hexosamine, fucose and sialic acid) and thickening of basal membrane. Hyperglycemia further accelerates synthesis of glycoporteins (Senthilkumar and Subramanian, 2008). In our study, administration of CA decreased glycoprotein content in diabetic rats which could be due to decreased hyperglycemic state and increased plasma insulin. Result on isolated psoas muscle indicated that CA (25 and 50 mg/kg) enhanced the uptake of glucose in muscle tissues in a short time of 30 min in absence of insulin (20% and 28% respectively) and maximum of 48% uptake by CA 50 mg/kg in presence of insulin. Increased glucose uptake in presence of insulin suggests increased binding of insulin to receptors and enhanced utilization of glucose in blood (Gupta et al., 2005).

Thus, the antidiabetic effect of CA could be mediated via several mechanisms: inhibition of α glucosidase activity; impaired glucose absorption; increased glucose uptake; modification of hepatic glycolgenic enzymes and insulinogenic activity.

From this study, we can conclude that chebulic acid has beneficial effects on blood glucose level. It has the potential to impart therapeutic effect in diabetes. Further pharmacological and biochemical studies are in progress to elucidate the mechanism of action of CA in detail at molecular level.

## 5. REFERENCES


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