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EVALUATION OF MECHANISM OF ANTI-DIABETIC ACTIVITY OF *TERMINALIA CHEBULA* ON ALLOXAN AND ADRENALINE INDUCED DIABETIC ALBINO RATS

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ABSTRACT

Diabetes is one of the major causes of premature disease and death worldwide, with wide prevalence in low and middle income countries like India. Deficiencies in diagnosis and treatment, especially cost factors contribute to the extent of the problem in developing countries. Herbal products are widely available and cost effective, hence we wanted to evaluate the antidiabetic action of ethanolic pulp extract of a locally available plant, *Terminalia chebula* (AETC) on alloxan induced diabetic rats in comparison to Glibenclamide along with the mechanism of such action. Five groups of albino rats, with six rats in each group were taken for the study. Diabetes was induced in group C, D and E by injecting alloxan 150mg/kg body weight intra-peritoneally. Group A and B received normal saline (5ml/kg/day orally) and AETC (100mg/kg/day orally) respectively for 4 weeks. Groups C, D and E received Normal saline (5ml/kg/day orally), AETC (100mg/kg/day orally) and Glibenclamide (5mg/kg/day orally) respectively for 4 weeks. Fasting blood glucose levels were estimated at the end of 1st, 2nd, 3rd and 4th week. For mechanism of action, glycogen content of liver, heart and skeletal muscle were estimated in alloxan induced rats and action of AETC on blood glucose in adrenaline-induced hyperglycemic rats was evaluated, using normal control, diabetic test (AETC) and diabetic standard (Glibenclamide) groups. Statistical analysis was done using one way ANOVA followed by Dunnet's multiple comparison test. Diabetic test and standard groups were compared by Unpaired 't' test. A *P* value of < 0.01 was considered significant. *Terminalia chebula* showed significant anti-hyperglycemic effect (*P*<0.01) without hypoglycemic action in normal rats, and efficacy was lower than glibenclamide in alloxan model but higher in adrenaline induced model. The glycogen content of liver significantly increased in diabetes induced albino rat which may be due to insulin like action of ingredients present in *Terminalia chebula. T. chebula* also showed reduction in blood glucose level on adrenaline induced hyperglycemia resulting from inhibition of α_2 receptor of pancreatic β-cells, thus promoting further insulin release.The ethanolic pulp extract of *Terminalia chebula* fruit has significant anti-diabetic activity probably due to insulin like action of its constituents and promotion of insulin release.

KEYWORDS: **Anti-diabetic activity, Ethanolic extract, *Terminalia chebula***

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INTRODUCTION

Diabetes mellitus comprises a group of common metabolic disorders characterized by hyperglycemia, which may be due to reduced insulin secretion, decreased glucose utilization and/or increased glucose production.[1] Diabetes is one of the major causes of premature disease and death, being part of the spectrum of non-communicable diseases that contribute to 60% of deaths worldwide. While the global prevalence of diabetes was estimated to be 6.4%, that is, about 285 million people in 2010 by the World Health Organization (WHO), the prevalence is projected to increase to 7.8% or 438 million of the world's population by 2030. 70% of these patients occur in low- and middle income countries. With an estimated 50.8 million people living with diabetes, India has the world's largest burden of this disease.[2] Deficiencies in both diagnosis and treatment are responsible for this situation. Moreover, treatment is costly and depends on the economic status of the diabetics and their families, especially in developing countries like India where health insurance and social security systems are not accessible to the vast majority. In this scenario, herbal medicine is advocated by WHO due to the low cost and easy availability.[3]More than 400 different plants and plant extracts have been described as reputedly beneficial for the diabetic patient. Many of these plants have been claimed to possess hypoglycemic properties but most claims are anecdotal and few have received adequate medical or scientific evaluation.[4]

Terminalia chebula is a tree belonging to the family Combretaceae occurring throughout India and also in Nepal, Sri Lanka and Burma. It is commonly known as black myroblans in English and grows mostly in deciduous forests up to about 1000 meters above sea level.[5] It is traditionally used in the treatment of a wide range of disorders including gingivitis, stomatitis, asthma, cough, dyspnea, dyspepsia, gastroenteritis, ulcers, diarrhoea, constipation, irritable bowel syndrome, hemorrhoids, candidiasis, malabsorbtion syndromes, hepatomegaly, splenomegaly, ascites, vesicular and renal caliculi, urinary discharges, tumors, skin diseases, leprosy, intermittent fever, rheumatism, arthritis, gout, neuropathy, paralysis, memory loss, epilepsy, depression, leucorrhea, diabetes, cardiovascular disease, anorexia and wounds.[6] Significant anti-diabetic activity of *T. chebula* fruits and seeds in albino rats was described earlier. [7,8] We wanted to evaluate the anti-diabetic activity of fruit pulp of *Terminalia chebula* on albino rats at doses lower than used previously for demonstrating anti-diabetic activity. Our objectives were:

1. To evaluate the anti-diabetic activity of fruit pulp of *Terminalia chebula*
2. To evaluate the mechanism of anti-diabetic activity of fruit pulp of *Terminalia chebula*

MATERIALS AND METHODS

We did a prospective interventional study in the department of Pharmacology, with technical help from the department of Biochemistry of Assam Medical College and Hospital, Dibrugarh, Assam, India. Ethical clearance was taken from the institutional animal ethics committee (634/02/A/CPCSEA, dated 19/05/2002).

Drugs

Drugs used were:
Dried fruit pulp of *Terminalia chebula.*
Glibenclamide tablets from Sanofi Aventis India, Mumbai.
Alloxan monohydrate obtained from Sigma Aldrich India, Bangalore.
Inj Adrenaline hydrochloride
Normal (0.9%) saline as vehicle

Preparation of T.chebula fruit extract

Terminalia chebula fruits obtained from the plant were botanically authenticated by Ms Belinda Lahon, PhD in Botany, University of North Bengal. As per the method of percolation described by Remington,[9] they were washed thoroughly and then air-dried on a drier table at room temperature. Then the dried pulps were

crushed in an electrical mixer-grinder. Five hundred grams of the powdered pulp were soaked in sufficient quantity of 90% ethyl alcohol and allowed to stand for 15 minutes. The soaked powder was then transferred to a percolator. The ground drug was packed firmly in the percolator and allowed to macerate for 24 hours at room temperature. Percolation was allowed slowly after 24 hours of maceration. The same procedure was repeated twice over the next twenty four hours till no further extraction was possible. The residue obtained from percolation was put in a vaccum desiccator. Twenty grams of a sticky black coloured alcoholic extract of *Terminalia chebula* fruit pulp (4% dry weight) was obtained. The suspension was used in a dose of 100 mg/kg BW for the respective groups as per previous studies in other models of diabetes. It was dissolved in normal saline and was used in the experiment according to the method of Satyavati, *et al*. [10]

Experimental Animals

Healthy adult albino rats (*Rattus norvegicus*) of either sex weighing approximately 150-200 gm were procured from Central animal house, Assam Medical College, Dibrugarh and provided standard laboratory diet with water *ad libitum*. They were kept in clean and dry cages and maintained in well-ventilated departmental animal house with 12 h light: dark cycle. All the animal experiments were conducted according to guidelines recommended by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). [11]

1. Evaluation of anti-diabetic activity

Induction of Experimental Diabetes

The animals were subjected to overnight fasting and diabetes was induced in the diabetic control, test and standard groups by a single intra-peritoneal injection of alloxan monohydrate at a dose of 150mg/kg body weight.[12,13] The fasting blood glucose level was determined after 72 hours of alloxan administration. Albino rats having blood glucose level equal to or more than 200mg% after 72 hours of administration of alloxan were selected for this study.

Experimental design

We evaluated the anti-diabetic action of alcoholic extract of pulp of *Terminalia chebula* (AETC) on alloxan induced diabetic albino rats.

For evaluation of anti-diabetic action, the animals included in the experiment were divided into five groups with six animals each and drugs were administered orally for four weeks as follows:

Group I (Normal control group): Normal saline 5ml/kg/day

Group II (Normal test group): AETC in normal saline 100mg/kg/day

Group III (Diabetic control group): Normal saline 5ml/kg/day

Group IV (Diabetic test group): AETC in normal saline 100mg/kg/day

Group V (Diabetic standard group): Glibenclamide in normal saline 25mg/kg/day

Fasting blood glucose levels were estimated at the end of 1st, 2nd, 3rd and 4th weeks.

Method of collection of blood and blood glucose estimation

Blood samples were collected from orbital sinus under light ether anesthesia, by capillary tube, following engorgement of retro-orbital sinus by pressing the jaw behind by the thumb. The capillary tube was inserted through medial canthus into the retro-orbital plexus with gentle rotation for free flow of blood.[14]

Blood glucose estimation was done by glucose oxidase method using glucose kit, as per the method of Sood, *et al*. [15]

2. Evaluation of mechanism of anti-diabetic activity

Induction of Experimental Diabetes

The animals were subjected to overnight fasting and diabetes was induced in the diabetic control, test and standard groups by a single intra-peritoneal injection of alloxan monohydrate at a dose of 150mg/kg body weight. Albino rats having blood glucose level equal or more than 200mg% after 72 hours of administration of alloxan were selected for the study.

Experimental design for glycogen estimation in alloxan diabetes

Rats were divided into four groups of six animals each as follows:

Group A (Normal control): Normal saline (5ml/kg/day orally).

Group B (Diabetic control): Normal saline (5ml/kg/day orally).

Group C (Test): *T.chebula* extract at a dose of 100mg/kg/day orally.

Group D (Standard): Glibenclamide at a dose of 0.5mg/kg.

After seventy two hours of alloxan administration, we administered the drugs to the animals in the respective groups. After two hours, we sacrificed the animals by decapitation and performed glycogen estimation of rat liver, skeletal muscle and cardiac muscle as per the methods of Carroll, *et al*.[16]

The calculation of glycogen was done as follows

$$\frac{DU}{DS} \times 0.1 \times \frac{\text{Volume of extract}}{\text{Weight (g) of tissue}} \times 100 \times 0.9 = \text{mg of glycogen per 100 g of tissue}$$

where,

DU = optical density of the unknown.

DS = Optical density of the standard.

0.1 = mg of glucose in 2 ml of standard solution.

0.9 = factor for converting glucose value to glycogen value.

Experimental design for adrenaline induced hyperglycemia

We evaluated the effect of *T.chebula* on adrenaline induced hyperglycemia by the methods of Gupta, *et al*,[17] and Anturlikar, *et al*,[18] by dividing the animals into groups of six numbers each, recording baseline fasting blood glucose and treating them as follows:

Group A: Normal saline at a dose of 5ml/kg.

Group B: *T.chebula* extract at a dose of 100mg/kg.

Group C: Glibenclamide at a dose of 0.5 mg/kg.

One hour after administration of drugs, animals were injected with 100 micrograms of intraperitoneal injection of adrenaline hydrochloride and blood glucose was estimated after 30 minutes.

Statistical analysis

The data were statistically analyzed using one way ANOVA (Analysis of Variance) test followed by Dunnet's multiple comparison test. Diabetic Test and standard groups were compared by Unpaired 't' test. A *P* value < 0.01 was considered significant. Data were presented as mean±SEM.

RESULTS

Results of anti-diabetic activity are summarized in Table 1 and Fig1, showing mean blood glucose level in different groups. In the diabetic test and standard groups, the percentage of reduction of blood glucose level was 28.1% and 34.37% respectively at the end of the first week. At the end of the second week, percentage of reduction was 41.8% and 55.12% and at the end of the third week, it was 66.4% and 73.62% in the respective groups. At the end of the fourth week of treatment, percentage of reduction was 62.9% and 70.9% respectively. These results are summarized in Fig 2.

Table 1

Effects of the ethanolic extract of Terminalia chebula on blood glucose levels (mg/dl) at 1, 2, 3 and 4 weeks from administration of alloxan in albino rats in diabetic control, test and standard groups

Group	Base line	72 hr after diabetes induction	1st week	2nd week	3rd week	4th week
A	97±.45	98±2	96±2	97±2	98±2	97±2
B	98±0.3	97±0.8	97±0.9	98±0.5	97±0.8	98±0.8
C	96±.45	280±.74[a]	320±5.8[a]	361±1.1[a]	417±0.9[a]	324±1.2[a]
D	97.5±0.2	270±.74[b]	230±3.2[b]	210±1.4[b]	140±2.6[b]	120±1.8[b]
E	96.5±0.2[c]	240±.91[b,c]	210±2.9[b,c]	162±2.9[b,c]	110±2.9[b,c]	94±1.9[b,c]

Values are expressed as mean±SEM (n = 6)
a = P<0.01 when compared to the normal control group
b = P<0.01 when compared to the diabetic control group
c = P<0.01 when the diabetic test and standard groups are compared. P<0.01 is highly significant.

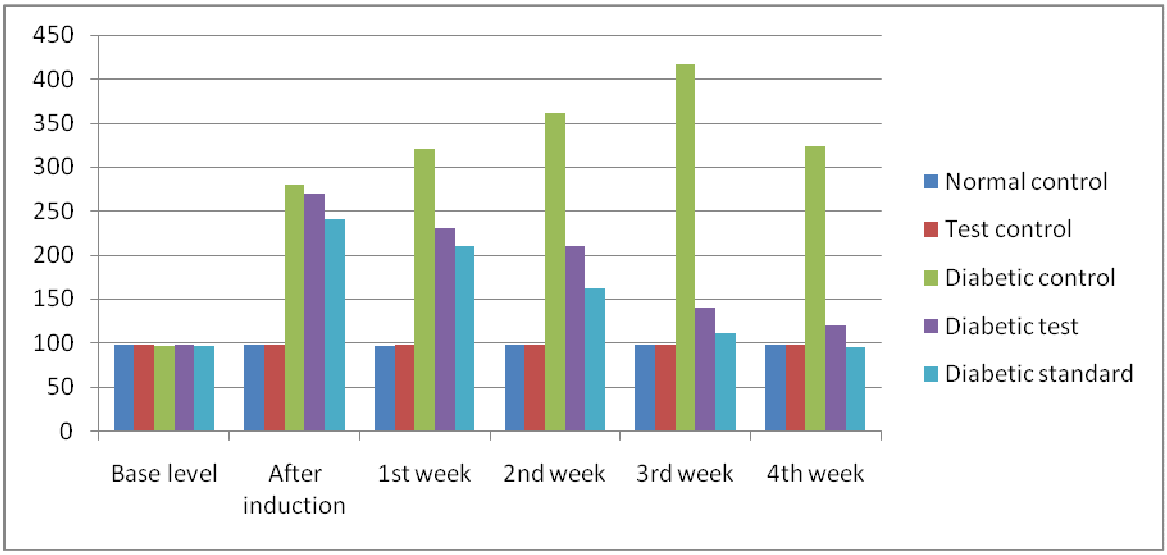

Figure 1

Effects of the ethanolic extract of Terminalia chebula on blood glucose levels (mg/dl) at 1, 2, 3 and 4 weeks from administration of alloxan in albino rats in diabetic control, test and standard groups

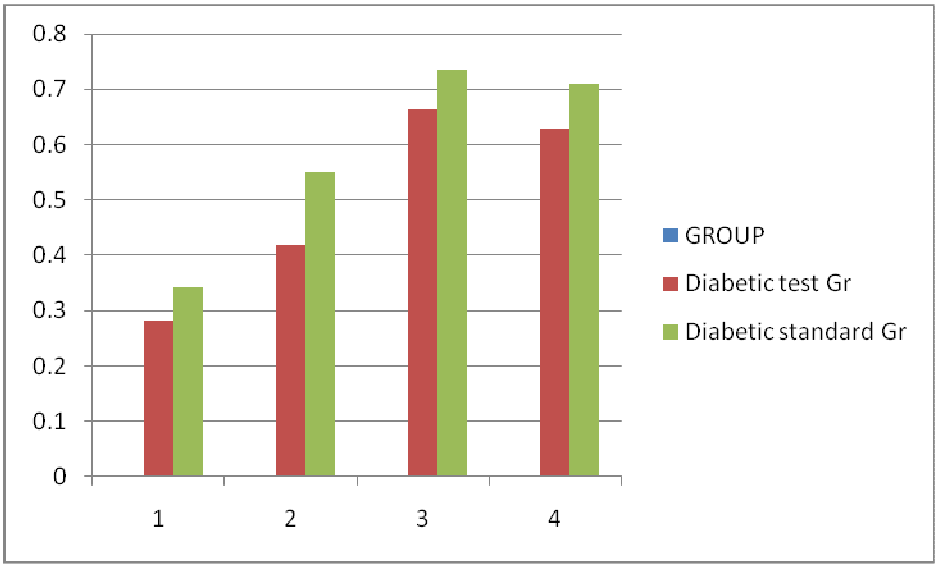

Figure 2

Percentage reduction in blood glucose in diabetic test and standard groups at the end of 1st, 2nd, 3rd and 4th week of administration of alloxan.

Table 2 and Fig 3 show the glycogen concentration in liver, skeletal muscle and cardiac muscle after treatment with extract of *T.chebula*. Compared to the diabetic control group, glycogen content of skeletal muscle and cardiac muscle of diabetic albino rats were found to be increased in *Terminalia chebula* group, but it was statistically insignificant. However, there was significant increase in liver glycogen in *Terminalia chebula* group compared to the diabetic control.

Table 3 and Fig 4 show the effect of *Terminalia chebula* on adrenaline induced hyperglycemia. The average rise in blood glucose level after adrenaline was 80 mg% in the control group as compared to 42mg%, 54mg% in animals treated with test and standard drugs. The percentage reduction of blood glucose by *T.chebula* was 48% and was found to be statistically significant compared to standard ($P<0.01$).

Table 2

Effects of T.chebula extract on Glycogen concentration (mg/100g) in liver, skeletal muscle and cardiac muscle after induction of alloxan diabetes in albino rats

Group	Liver	Skeletal muscle	Cardiac Muscle
Gr A	90±1.2	40±0.4	44±2.8
Gr B	30±2.9[a]	20±1.5[a]	30±2.2[a]
Gr C	75±1.7[b]	21±1.2	32±1.4
Gr D	85±2.9[b,c]	30±1.5[c]	40±1.1[c]

Values are expressed as mean±SEM.
a = P<0.01 when compared to the normal control group.
b = P<0.01 when compared to the diabetic control group
c = P<0.01 when the diabetic test and standard groups are compared.

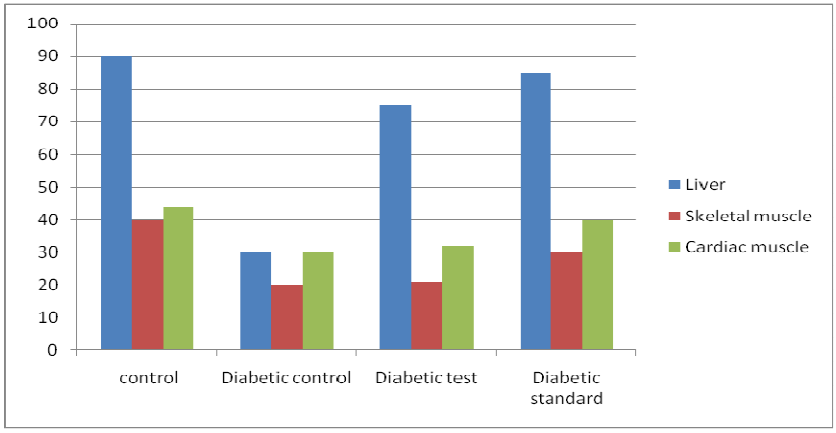

Figure 3

Effects of T.chebula extract on Glycogen concentration (mg/100g) in liver, skeletal muscle and cardiac muscle after induction of diabetes in albino rats

Table 3

Effect of T.chebula extract on blood glucose levels (mg/dl) following intraperitoneal injection of adrenaline hydrochloride in albino rats

Group	0hr	30min after Adrenaline	Change	%increase (mg)	%decrease (mg)
Gr A	100±0.9	180±0.9	80±1.3	80	
Gr B	101±0.5	143±0.6[a]	42±1[a]	41.6	48
Gr C	102±0.9	156±0.9[a,b]	54±1.7[a,b]	52.9	33.8

Values are expressed as mean±SEM

a = P<0.01 when compared to the control group
b = P<0.01 when the diabetic test and standard groups are compared.

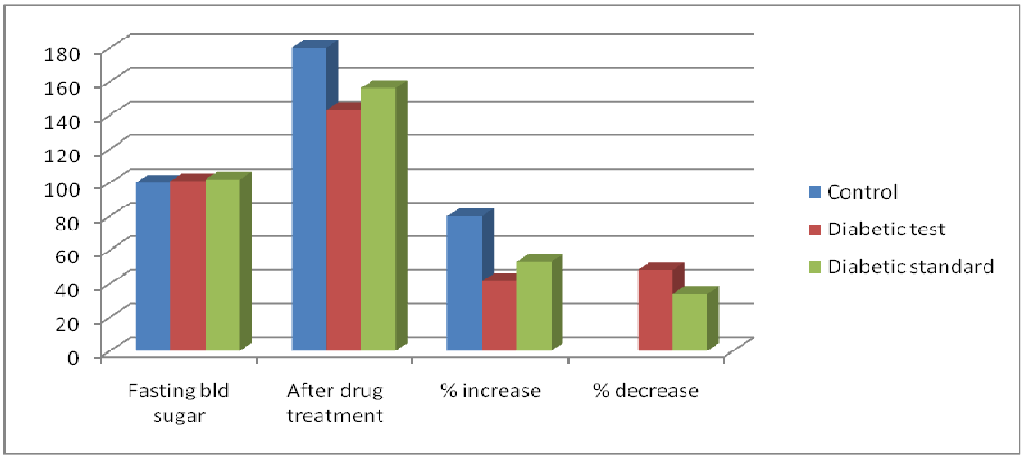

Fig 4

Effect of T.chebula extract on blood glucose levels (mg/dl) following intraperitoneal injection of adrenaline hydrochloride in albino rats with percentage reduction in blood glucose

DISCUSSION

Our aim was to evaluate whether the fruit pulp of *Terminalia chebula* had anti-diabetic activity and to study the mechanism of such anti-diabetic effect, if present. Alloxan - induced hyperglycemia in rodents is a widely used screening model for testing anti-diabetic activity.[19]Alloxan rapidly binds to or accumulates in pancreatic B-cells as distinct from non-B cells. The selective uptake of this cytotoxic agent by the insulin-producing B-cells might account for its well-known diabetogenic effect.[20]The underlying mechanism of alloxan is still a matter of debate. It probably exerts its diabetogenic effect by the production of hydrogen peroxide in intact islets.[21]Alloxan and the product of its reductions, dialuric acid establishes a redox cycle with the formation of superoxide radicals. These radicals undergo dismutation to hydrogen peroxide. Thereafter highly reactive hydroxyl radicals are formed by the Fenton reaction. The action of reactive oxygen species with a simultaneous massive increase in cytosolic calcium concentration causes rapid destruction of B cells.[19]

We found that the ethanolic extract of fruit pulp of *Terminalia chebula* lowered blood glucose levels in alloxan induced diabetic rats. The extract administered at the dose of 100mg/kg/day orally for four weeks produced statistically significant ($P<0.01$) reduction in blood glucose level in diabetic albino rats as compared to diabetic control. However, there was no difference between normal control and normal test group after four weeks of treatment. This proves that *Terminalia chebula* is anti-hyperglycemic but has got no hypoglycemic action. Glibenclamide at the dose of 25mg/kg/day orally for four weeks reduced blood glucose level in diabetic rats significantly ($P<0.01$). The effect of the test drug at the dose of 100mg/kg/day on blood glucose levels was significantly ($P<0.01$) less than that of the standard drug glibenclamide. An earlier study had found that the methanolic extract of *Terminalia chebula* inhibits lipid peroxide formation and scavenge hydroxyl and superoxide radicals in diabetic rats confirming its anti-diabetic potential.[22]A study which evaluated the anti-diabetic potential of *Terminalia chebula* also found that oral administration of ethanolic extract of the fruits (200mg/kg/day) for 30 days significantly reduced the levels of blood glucose and glycosylated hemoglobin in streptozotocin induced diabetic rats.[7] Another study, which investigated the antidiabetic activity of chloroform extract of *Terminalia chebula* seed powder in streptozotocin induced diabetic rats using short term and long term study protocols found that the extract significantly reduced the blood glucose level.[8] In another study, it was seen that water extract of *Terminalia chebula* at a dose of 200mg/kg body weight improved glucose tolerance and reduced fasting blood glucose in streptozocin induced diabetic rats.[23] In a recent study, the anti-diabetic activity of three different doses of *T. chebula* fruit extract – 50mg/kg, 100mg/kg and 200mg/kg body weight was evaluated in the rat model of metabolic syndrome. It was seen that *T. chebula* fruit extract exerts a significant and dose-dependent glucose lowering effect when rats were treated with it after being fed a high fructose diet. [24] Thus, our findings were similar to those of previous studies, with demonstration of highly significant anti-diabetic activity at a dose of 100mg/kg, which was lower than the dose used in earlier studies. The anti-diabetic activity was less than the standard oral hypoglycemic glibenclamide.

Glycogen content of skeletal muscle and cardiac muscle of diabetic albino rats was found to be increased by *Terminalia chebula*, but it was statistically insignificant. However, extract of *Terminalia chebula* produced significant increase in liver glycogen. The increase in glycogen concentration in liver may be due to insulin like action of the ingredients present in *Terminalia chebula.* Insulin activates the enzyme glycogen synthase while inhibiting glycogen phosphorylase responsible for glycogenolysis in liver and muscle.[25] Insulin deficiency in diabetes results in reduced glycogen in liver and muscle. Ingredients present in *Terminalia chebula* caused an increase in glycogen concentration of the liver probably by stimulating the enzymes glycogen

synthase and hexokinase to increase glycogen synthesis.[7] The increase in liver glycogen may also have been brought about by inhibition of glucose-6-phosphatase leading to accumulation of glucose-6-phosphate, which allosterically inhibited the enzyme glycogen phosphorylase.[26] Diminished phosphatidylinositol 3-kinase (PI-3K) activation in diabetes as a result of insulin deficiency has been associated with impaired skeletal muscle glycogen synthase enzyme.[27] Ethanolic extract of *T.chebula*, due to the insulin-like action of its ingredients, probably increased PI-3K activation leading to stimulation of muscle glycogen synthase. The increased concentration of glycogen in skeletal and cardiac muscle could also be attributed to increased expression and translocation of GLUT-4 glucose transporters as a result of increased PI-3K activation, leading to increased peripheral uptake of glucose.[28]In another study, hepatic and skeletal muscle glycogen content decreased in diabetic controls, these alterations were partly prevented in the group treated with aqueous extract of *T. chebula*, when compared to the healthy controls.[29] Senthil Kumar, *et al* also found that the presence of biologically active ingredients in the fruit extract of *T.chebula* potentiates its anti-diabetic properties by regulating mitochondrial enzymes, which become dysfunctional in diabetes. [30] However, effect of *T.chebula* extract on glycogen concentration in liver, skeletal muscle and cardiac muscle is significantly lower compared to glibenclamide.

Evaluation of the effect of ethanolic extract on adrenaline induced hyperglycemia revealed that *Terminalia chebula* did cause appreciable reduction of the hyperglycemic response induced by adrenaline. The average rise in blood sugar after adrenaline was 80mg% in control, 42mg% in test drug and 54mg% with the standard drug. Thus, the test drug was better in preventing hyperglycemia than glibenclamide in adrenaline induced model. Adrenaline produces hyperglycemia by inhibiting insulin release, stimulating glycogenolysis in muscle (thus providing substrate in the form of lactate for hepatic gluconeogenesis), stimulating glucagon secretion and stimulating ACTH secretion which, in turn, stimulates glucocorticoid secretion from the adrenal cortex[31] It has also been reported that adrenaline produces hyperglycemia by increasing glucose uptake from both the large and small intestine[32] *Terminalia chebula* probably prevented the rise in blood sugar by inhibiting the adrenaline induced stimulation of α_2 receptors present on pancreatic beta cells, thus helping insulin release.[33] On comparing *T.chebula* extract and glibenclamide, *T.chebula* was found to have significantly better effect in preventing adrenaline induced hyperglycemia than glibenclamide.

Other researchers have found that the antihyperglycemic effect of *Terminalia chebula* may be due to the presence of phytochemicals such as tannin.[34] Oxidative stress or excessive production of reactive oxygen species is being implicated in many diseases including diabetes.[35] It is an excellent free radical scavenger, the property arising mainly from the presence of well-known antioxidants like ascorbate, gallic acid and ellagic acid.[36] One of its constituents chebulagic acid was found to have alpha-glucosidase inhibiting activity.[37] Some observers have found that inhibitory effect on carbohydrate hydrolyzing enzymes like alpha-amylase of tannins isolated from the alcoholic extract of fruits of *Terminalia chebula* is comparable to the alpha glucosidase inhibitor, acarbose which is an effective anti-diabetic agent. [38]

CONCLUSION

In conclusion, the present study shows that the alcoholic extract of *T.chebula* fruit pulp has anti-hyperglycemic action in alloxan induced diabetic rats and the effect is less than that of glibenclamide in alloxan model but higher in adrenaline induced model. It probably exerts its anti-hyperglycemic action by insulin like action of its ingredients as well as by promoting insulin release in response to hyperglycemia in addition to other mechanisms. It needs clinical evaluation for demonstration of efficacy and safety in humans. Hence, *Terminalia chebula* may have a promising role in the management of diabetes mellitus especially in a country like

India where conventional treatment is not easily accessible to the general population due to cost factors.

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