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Evaluation of Pharmacological Potential of *Tamarindus indica* in Streptozotocin Induced Diabetic Rats

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ABSTRACT

Diabetes mellitus is associated with dyslipidemia. The present study was carried out to evaluate the antidiabetic and hypolipidemic potential of hydro-alcoholic and aqueous seeds extract of Tamarindus indica on Streptozotocin induced diabetic rats. In oral glucose tolerance test the blood glucose level was significantly ($P<0.05$) controlled in 0-90 min after glucose administration in normal and streptozotocin induced diabetic rats. The blood glucose level of Streptozotocin induced diabetic rats were determined at 0,7,14 days of treatment after 14 days of treatment blood glucose levels were significantly ($P<0.05$) decreased. Hydro-alcoholic seeds extract at the dose of 200 mg/kg significantly increased the serum insulin level. Tamarindus indica seeds extracts at all the dose level 100 and 200 mg/kg, after 14 day days of treatment significantly ($P<0.05$) increased hepatic glycogen level. On the other hand after 14 days of treatment lipid profile cholesterol (CHOL), high density lipoprotein (HDL), triglyceride (TG), low density lipoprotein (LDL), very low density lipoprotein (VLDL) were significantly ($P < 0.05$) improved in diabetic rats treated with Tamarindus indica seeds extracts. The results suggested that the both hydro-alcoholic and aqueous extracts of Tamarindus indica seeds possess potential antidiabetic, hypolipidemic effect.

Key words: Diabetes mellitus, hypolipidemic, *Tamarindus indica*, insulin, streptozotocin.

1. INTRODUCTION

Now a day's researchers are working more for searching a potential anti-diabetic agent from plant source.[1] From pre-historic period people all over the world are depending on nature for traditional healers.[2] The World Health Organization (WHO) has recommended the use of traditional plants for treatments of diabetes mellitus as they are cost effective, free of toxicity, with very less or no side effects.[3]

India is well rich with medicinal plants. Knowledge of medicine use of medicinal plants in India is amassed over millennia by tribal. For thousands of years Indian plants have been attracting attention of foreign countries. People from countries like China, Cambodia, Indonesia, Baghdad, used to come to ancient universities of India like Takshila (700 B.C.) and Nalanda (500 B.C.) to learn health science of India.[4]

Diabetes mellitus is one of the oldest known disease. In ancient scripture it was reveal that as early as 700-200 B.C. Sushruta used the term "Madhumeha" for diabetes mellitus in 6th Century. He describes it as a disease of rich, produced by over consumption of rice, flour and sugar.[5] By definition, the term diabetes mellitus describes the metabolic disorder having heterogeneous etiologies which are characterized by chronic hyperglycemia and abnormal of carbohydrate, protein and fat metabolism due to impaired in insulin secretion, resistant to insulin action, or both.[6]

In addition to hyperglycaemia, In type 2 diabetic persons increase chances of metabolic cardiovascular risk factors, because there is altered in lipid profile occur which is characterized by elevated levels of circulating free fatty acids (FFAs) and triglycerides (TGs), a decrease in high-density lipoprotein cholesterol (HDLC) along with excess fat deposition in various tissues including the liver.[7]

Tamarind (*Tamarindus indica* L.) is a tree-type of plant which is indigenous to tropical Africa but has become naturalized in North and South America from Florida to Brazil, and is also cultivated in subtropical China, India, Pakistan, Indochina, Philippines, Java[8,9]. *Tamarindus indica* is a large tree 12-18 m. high, branches spreading belonging to family: Caesalpiniaceae, Leaves: 5-12.5 cm long, rachis slender channeled, stipulus linear, cadcous. Leaflets: Subsessile, 10-20 pairs, tolerably closely set on the rachis, 8-30 by 5-8 mm. oblong, obtuse, glabrous reticulately veined. It is a dicotyledonous plant. Seed coat is brownish black in colour though the kernel is white in colour.[10]

Tamarindus indica was used as a traditional medicine for the management of diabetes mellitus.[11] In the present study hydroalcoholic and aqueous extract of *Tamarindus indica* seeds have been evaluated for antihyperglycemic, hypolipidemic and insulin secretory activity using experimental animal model (rats).

2. MATERIALS AND METHODS

2.1 Plant material

Tamarindus indica seeds were collected from local market of Raipur, Chhattisgarh, India. Seeds were washed under running tap water followed by rinsed with distilled water for five minutes. Plant as well as seed of *Tamarindus indica* were identified and authenticated by Dr. P. C. Panda, Principal scientist taxonomy division "Regional Plant Resource centre, Bhubaneswar, Odisha, India". A voucher specimen was also deposited (Voucher specimen no: BM -1) in the institution for further reference.

2.2 Drugs and Chemicals

Gluco-One glucose measurement strips (Dr. Morpene), One Touch blood glucometer, LINCO ELISA kit, Lipid profile kits were procured from (Span Diagnostics, Surat, India). Glibenclamide was obtained as a gift sample from (Bioplus life sciences, Bangalore, India). Streptozotocin and the rest of the chemicals utilized were of analytical grade and were purchased from Fisher scientific, Mumbai, India.

2.3 Preparation of Plant extracts

The dried seeds were coarsely powdered and passed through sieve No.20. The aqueous extraction was carried out by boiling 500 g powdered with purified water for 10 h. Hydroalcoholic extraction was carried out by mixing the (500 g) powder with ethanol and water (8:2) for 2 days. The resulted extracts were filtered through Whatman filter paper no. 4 and concentrated by rotary evaporator under reduced pressure and low temperature. The yield of extract was 9.24% (w/w).[12]

2.4 Animals

Studies were carried out using Wistar albino rats of either sexes (150-200 g). They were housed in standard polypropylene cages at room temperature 23 ± 2 °C and 50 ± 5% relative humidity; under a light/dark cycle of 10/14 h. Rats were acclimatized for a period of one week before the initiation of experiment. Animals were provided with standard rodent pellet diet (Amrit feeds, Raipur, Chhattisgarh. India) and water *ad libitum*. The animals were deprived of food for 24 hours before experimentation, but had free access to drinking water. Experimental protocol was approved by Institutional Animal Ethics Committee (IAEC), SBRL, Bhopal (Approval no. SBRL/IAEC/2012/11) which follows guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals). Normoglycemic animals were selected for this experiment having the fasting blood glucose at about the level 85 ± 5 mg/dl.

2.5 Acute oral toxicity study and selection of doses

Acute toxicity was carried out according to OECD guideline 423.[13]

2.6 Induction of diabetes

Freshly prepared solution of Streptozotocin (STZ) (50 mg/kg in 0.1 M ice cold citrate buffer $_{P}$H 4.5) was injected (i.p.) to overnight fasted rats. After 48h of STZ administration, fasting blood glucose levels were measured and blood glucose levels between 150 and 250 mg/dl were selected for study.[14]

2.7 Assessment of antidiabetic activity in normal rats

The animals were assigned into seven groups (I-VII) of six animals each and received the following treatments: Group I: Normal control received Tap water, Group II: Diabetic control (STZ 50 mg/kg) received Tap water, Group III: Diabetic + Glibenclamide (2.5 mg/kg) as standard drug, Group IV: Diabetic + hydroalcoholic extract of *Tamarindus indica* seeds HAETIS (100 mg/kg), Group V: Diabetic + HAETIS (200 mg/kg), Group VI: Diabetic + aqueous extract of *Tamarindus indica* seeds (AETIS)

(100 mg/kg) And Group VII: Diabetic + AETIS (200 mg/kg) for 14 days. The effects HAETIS and AETIS on STZ induced diabetic rats were determined weekly once by changes in body weight, blood glucose level measurement on overnight fasted rats.[14] At the end of the experimental period (13 days), the animals were deprived of food overnight and sacrificed by decapitation.

2.8 Estimation of Biochemical Parameters

2.8.1 Blood glucose level

Blood glucose analysis was done by GOD-POD method using Glucose Estimation Kit (Dr. Morpene, New Delhi, India).

2.8.2 Plasma insulin concentration

Serum insulin was estimated using insulin ELISA kit (*LINCO ELISA kit*) by ELISA reader.

2.8.3 Hepatic glycogen level

Hepatic glycogen content was determined according to the anthrone-H_2SO_4 methods, with glucose as the standard (Minzhu). Briefly, liver tissue (<100 mg) was homogenized in three volumes of an ice-cold 30% (w/v) KOH solution and dissolved in a boiling water-bath (100 ^oC) for 20 min. Glycogen was resolubilized in distilled water and the glycogen concentration was then determined by treatment with an anthrone reagent (2 g anthrone/1, l of 95% (v/v) H_2SO_4), and the absorbance was measured at 620 nm.[15,16]

Aliquot was then calculated using the following equation:

$$\mu g \text{ of glycogen in aliquot} = \frac{100xU}{1.11x\ S}$$

where U is the optical density of the unknown test solution and S is the optical density of the 100 µg glucose standard

2.8.4 Determination of lipid profile

High density lipoprotein cholesterol (HDLC), Total Cholesterol (TC), Triglyceride (TG) content in plasma was estimated by using a reagent kit, (Span Diagnostic Ltd. Surat India). Very low density lipoprotein cholesterol (VLDLC) and Low density lipoprotein cholesterol (LDLC) fractions were calculated as follows: VLDLC= Triglyceride/5 and LDL = (Total cholesterol) - (HDL Cholesterol) – (Triglyceride/5).

3. RESULTS

3.1 Acute oral toxicity study

In acute toxicity study, there was no any significant toxic symptom appears up to 2000 mg/kg so it was safe.

3.2 Effect of HAETIS and AETIS on oral glucose tolerance

HAETIS and AETIS were administered at the dose of (100 and 200mg/kg) 60 minutes prior to glucose loading. Blood glucose levels were measured at 0, 30, 60 and 90 min after glucose administration and AUC were determined. Animals treated with HAETIS and AETIS as well as glibenclamide received group considered as significant at the level of ($P<0.05$, $P<0.01$) respectively. Decrease of blood glucose level was observed (Figure.1).

3.3 Effect of HAETIS and AETIS on blood glucose level

The effects of repeated oral administration of HAETIS and AETIS on blood glucose level in STZ-diabetic rats at the dose of 100 and 200 mg/kg and standard glibenclamide for 14 days are presented in (figure.2). Blood glucose levels were measured at 1, 7 and 14th day of experiment and AUC was determined. Results indicated that there was significant ($P<0.05$) decreased of blood glucose level of HAETIS and AETIS treated groups were observed. Standard drug treated group also exhibited a significant ($P<0.01$) decrease of blood glucose level.

3.4 Effect of HAETIS and AETIS on serum insulin

Streptozotocin caused a significant decrease in serum insulin level in experimental rats. HAETIS and AETIS at the dose of 100 and 200 mg/kg, b.w. were administered for 14 days into different groups of animals. HAETIS at 200 mg/kg showed significant ($P<0.05$) increase in serum insulin level in diabetic animals (Figure.3).Standard drug (glibenclamide at the dose 2.5 mg/kg b.w) treated group showed a significant ($P<0.01$) increase in serum insulin level. In other groups there was no significant changes in insulin level was observed.

3.5 Effect of HAETIS and AETIS on hepatic glycogen level

Glycogen content was significantly decrease ($P<0.05$) in diabetic control group compared to normal control (Figure.4) Administration of HAETIS and AETIS at the dose of 100 and 200 mg/kg for 14 days exhibited significant increase in hepatic glycogen level ($P<0.05$) and standard drug Glibenclamide (2.5mg/kg) for 14 days showed significant ($P<0.01$) increase in hepatic glycogen level.

3.6 Effect of HAETIS and AETIS on lipid profile

Serum lipid profiles of rats were determined at the end of the experiment. It was observed that there was a significant (P<0.05) increase in serum Cholesterol, Triglyceride, LDL and VLDL and decrease of HDL level in STZ-induced group (Table.1). After treatment of HAETS, AETS at the dose 100 and 200 mg/kg for 14 days there was a significant decrease in serum cholesterol Triglyceride, LDL and VLDL level. The HDL level was also significantly (0.05) increased. In standard drug glibenclamide treated group there was significant (0.01) decrease of Cholesterol, Triglyceride, LDL VLDL and significant increase of HDL was observed.

3.7 Statistical Analysis

Results are expressed as Mean ± SEM. One-way analysis of variance (ANOVA), followed by Dunntt's multiple comparison test were carried out to determine the significant differences. Mean of negative control group and standard treated groups statistically significant if (P<0.01). Mean of negative control and HAETIS and AETIS treated groups statistically significant if (P<0.05). The analysis of the results was carried out using GRAPH PAD Prism 5.0 package software.

4. DISCUSSION

Tamarindus indica is a well-known traditional medicinal herb, Possesses diverse biological activities and pharmacological functions including reducing blood glucose level and serum lipids.[17] However, its pharmacological and chemical bases are not well understood.

In this study we have evaluated the antidiabetic and hypolipidemic activity of HAETIS and AETIS in streptozotocin induced diabetic rats. The result of OGTT performed in diabetic and normal rats demonstrated that hydroalcoholic and aqueous extracts of *Tamarindus indica* seeds decreased blood glucose level. The comparison was made with standard drug glibenclamide. STZ-induced diabetes is characterized by hypoinsulinemia and decreased body weight. Treatment with metformin or antidiabetic agents greatly improved polyuria and polydipsia, indicating improvement in the diabetic conditions.[18]

After induction of diabetes by STZ significant hyperglycemic state was achieved. Oral administration of HAETIS and AETIS for 14 days caused a significant decrease in blood glucose levels. The possible mechanism by which HAETIS and AETIS mediate antidiabetic effect may be by potentiating insulin secretion from existing pancreatic β-cells by signaling them which was evident by significant increase in insulin level in HAETIS extract treated animals. Hence, based on this experimental evidence, it can be stated primarily that the hydroalcoholic extract of seed of *Tamarindus indica* may contain some biomolecule(s) that may either sensitizes the insulin receptor for insulin or stimulate the β-cell of islets of langerhans in pancreas in STZ-induced diabetic rat that may restore plasma level of insulin there by re-establishment of normal blood glucose level was made possible. In diabetic rats body weight decreases.[19] Administration of *Tamarindus indica* seeds extract increases the body weight in STZ-induced diabetic rats observed in our earlier study.

Evidences suggest that diabetic rats showed an obvious decrease in insulin level. After 14 days treatment with T*amarindus indica* seeds extracts, the serum insulin and glycogen concentration significantly increased as compared with the STZ-induced untreated diabetic rats.

Streptozotocin shows β-cell cytotoxic effect by enhancing the production of the superoxide radical which can damage pancreatic β-cells. However, these pathological phenomena were ameliorated by the treatment of and HAETIS and AETIS which was in line with the significant increase in the plasma insulin levels following administration of the extracts. Therefore, the possible mechanism by which the HAETIS and AETIS brings about its antidiabetic effect may be by increasing the insulin level because of the protective effect of the extracts to pancreatic β-cells and stimulation of insulin secretion from the remaining pancreatic β – cells. [20]

Insulin is the regulator of glycogenesis in muscle and liver. Different researchers found that in diabetic condition that glycogen content decreases in muscle and liver. [21] The decrease in both muscle and hepatic glycogen observed in this study may be due to lack of insulin in the diabetic state and these results probably due to the inactivation of glycogen synthetase system. Supplementation of the HAETIS, AETIS for 14 days to diabetic rats resulted graded and significant elevation in liver glycogen level. This focus the one possible way of antidiabetogenic action of this extract by improvement of glycogenesis process in muscle and liver.

In addition to its antidiabetic effect HAETIS or AETIS were also able to improve some lipid metabolites including TC, TG, HDL and LDL cholesterol levels in diabetic rats. It has been reported that in diabetes alteration reported that diabetes are associated with profound alterations in lipid and lipoprotein profile.[22, 23] By regulating plasma lipid level macro and micro vascular complications can be regulated.[24] Thus, this result suggested that by preventing diabetes we can prevent dyslipidemia and diabetes related complications.

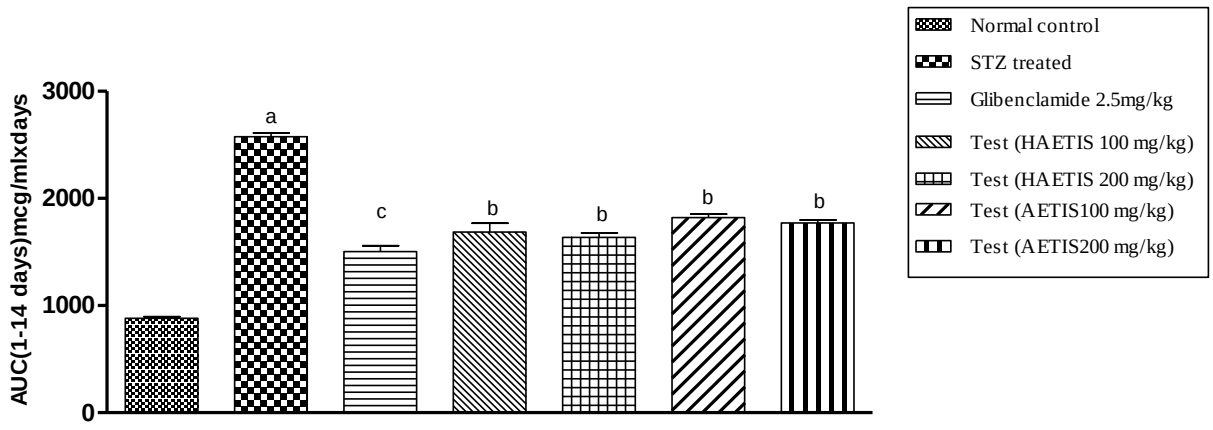

Figure 1: Effect of *Tamarindus indica* seeds extracts on oral glucose tolerance test (OGTT) in diabetic and normal albino rats. Each value is expressed as Mean ± S.E.M. (n=6).

[a]P<0.05 when compared to corresponding value of normal control.
[b]P<0.05 when compared to corresponding value of diabetic control.
[c]P<0.01 when compared to corresponding value of diabetic control.

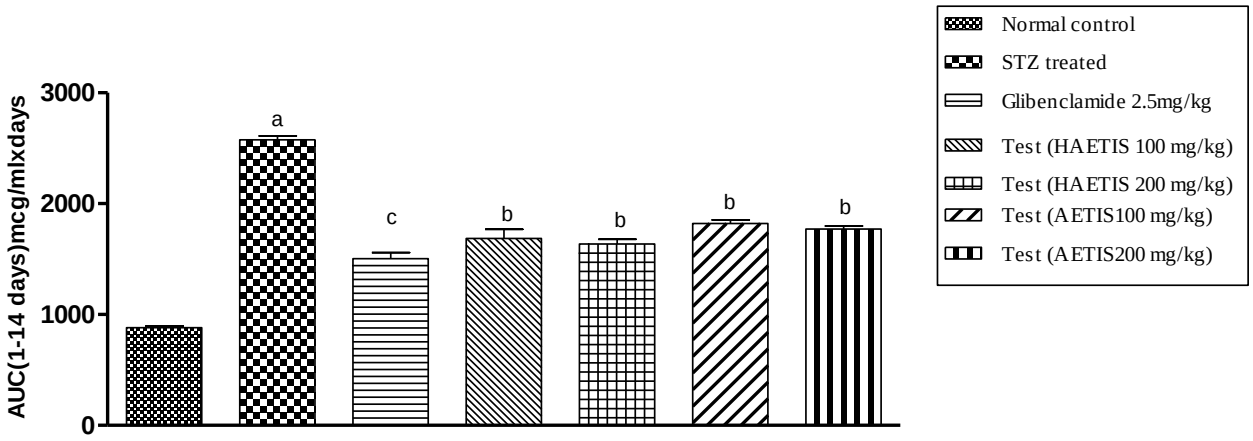

Figure. 2: Effect of *Tamarindus indica* seeds extract on blood glucose level in diabetic and normal albino rats. Each value is expressed as Mean ± S.E.M. (n=6).

[a]P<0.05 when compared to corresponding value of normal control.
[b]P<0.05 when compared to corresponding value of diabetic control.
[c]P<0.01 when compared to corresponding value of diabetic control.

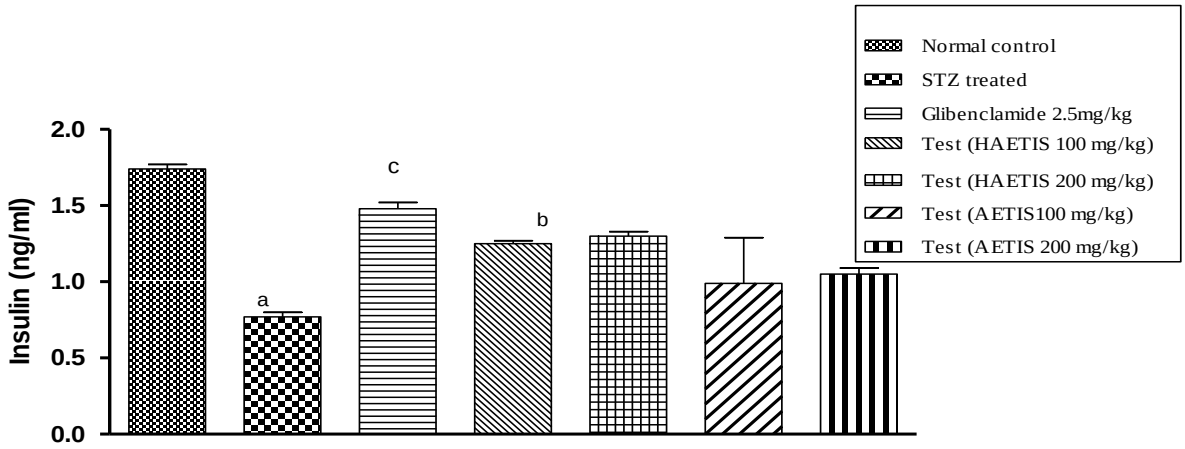

Figure. 3: Effect of *Tamarindus indica* seeds extracts on serum insulin level in STZ-induced diabetic and normal albino rats after 14 days of treatment. Each value is expressed as Mean ± S.E.M. (n=6).

[a]P<0.05 when compared to corresponding value of normal control
[b]P<0.05 when compared to corresponding value of diabetic control
[c]P<0.01 when compared to corresponding value of diabetic control

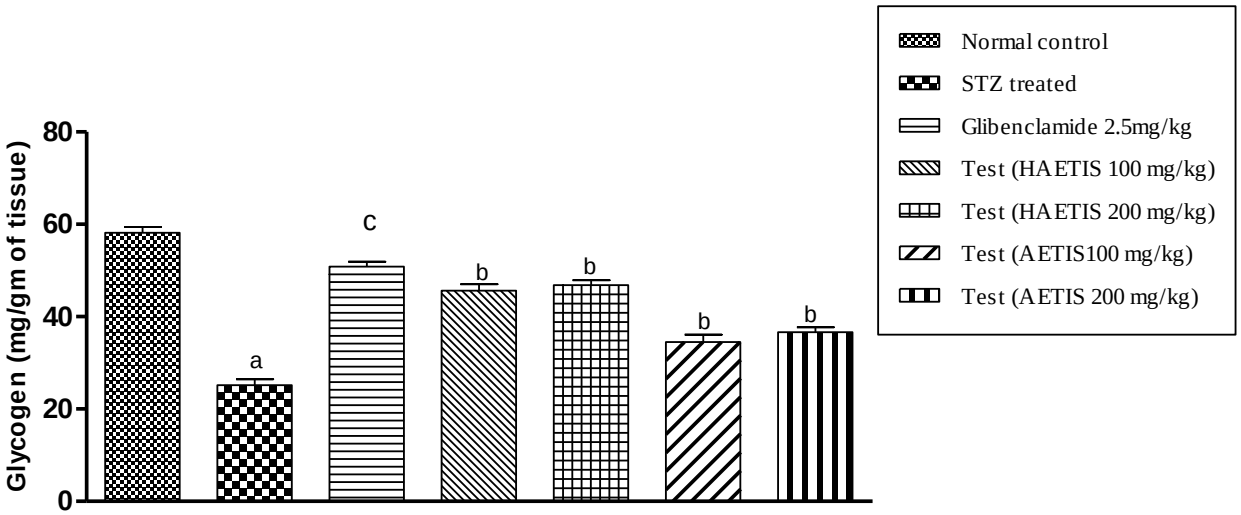

Figure. 4: Effect of *Tamarindus indica* seeds extracts on liver glycogen level in diabetic and normal albino rats after 14 days of treatment. Each value is expressed as Mean ± S.E.M. (n=6).

[a]P<0.05 when compared to corresponding value of normal control
[b]P<0.05 when compared to corresponding value of diabetic control
[c]P<0.01 when compared to corresponding value of diabetic control

Table 1: Effect of *Tamarindus indica* seeds extracts on serum level of Total Cholesterol, HDL cholesterol, Triglycerides TG, LDL Cholesterol and VLDL Cholesterol in diabetic and normal albino rats after 14 days of treatment

Groups	Treatment	CHL (mg/dl)	HDL (mg/dl)	TG (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
I	Normal Control (NC)	146.33 ±1.9	69.8±1.49	131±2.47	50.53±0.67	26.13±0.37
II	Streptozotocin (STZ)	212.83±1.77[a]	37.33±1.43[a]	199.66±2.47[a]	137.06±0.59[a]	39.93±0.34[a]
III	STZ+ Glibenclamide	166.16±1.1.79[c]	58±2.17[c]	159.33±2.45[c]	80.73±1.06[c]	32.1±0.33[c]
IV	STZ + HAETIS 100 mg/kg	180.33±2.76[b]	46.16±2.7[b]	177.16±1.4[b]	102.2±4.37[b]	35.8±0.28[b]
V	STZ+ HAETIS 200 mg/kg	178.83±4.26[b]	54.66±2.17[b]	172.5±2.97[b]	97.96±5.85[b]	34.03±0.61[b]
VI	STZ+AETIS 100 mg/kg	198.5±3.25[b]	47.5±2.06[b]	186.5±2.64[b]	121.5±1.42[b]	37.33±0.51[b]
VII	STZ+ AETIS 200 mg/kg	199.16±3.16[b]	50.33±1.14[b]	184.66±1.87[b]	117±0.84[b]	36.66±0.37[b]

Results are expressed as Mean ± S.E.M. (n=6), Evaluated by using one way ANOVA followed by Dunnett's multiple comparison test.
[a]P<0.05 when compared to corresponding value of normal control
[b]P<0.05 when compared to corresponding value of diabetic control
[c]P<0.01 when compared to corresponding value of diabetic control

After treatment with HAETIS, AETIS blood glucose, TC, TG, LDL, VLDL significantly decreased and at the same time cardio protective HDL lipid level was increased. This provides evidence in favor of the views that *Tamarindus indica* could play an important role in treating diabetic/hyperlipidemic patients. Which could prevent or helpful in reducing the complications of lipid profile seen in some diabetic in which hyperglycemia and hypercholesterolemia quite often coexist. Many current oral antidiabetic or hypolipidemic agents are synthetic drugs with certain adverse effect.[25] Our study reveals the potential of *Tamarindus indica* for use as a natural oral agent with both antidiabetic and hypolipidemic effect.

In summary, the present study has shown that HAETIS, AETIS demonstrated a clear antidiabetic and hypolipidemic effects in STZ-induced diabetic rats. The investigation helpful in understanding the mechanism of action of HAETIS, AETIS and reveal the potential for use as natural oral agent with both antidiabetic and hypolipidemic effects. Hypercholesteremia and hypertriglyceridemia are primarily responsible for the development of atherosclerosis and coronary heart disease which are the secondary complications of diabetes.[26] *Tamarindus indica* seeds and leaves extract significantly decreased serum triglycerides and total cholesterol, LDL and VLDL in STZ-diabetic rats. Thus, it is reasonable to conclude that *Tamarindus indica* seeds extract are having capacity to modulate lipid abnormalities in circulatory system.

4. CONCLUSION

From this study it can be concluded that hydroalcoholic and aqueous extracts of *Tamarindus indica* seeds has beneficial effects on antidiabetic and antihyperlipidemic potential in STZ-induced diabetic rat model. This was mediated via increase insulin secretion. Thus, lending support to its folkloric use in the management of diabetes mellitus.

5. CONFLICT OF INTEREST

The author declares that there is no any conflict of interest.

6. ACKNOWLEDGMENTS

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