

EVALUATION OF ANTI HYPERGLYCEMIC AND ANTI HYPERLIPIDEMIC ACTIVITY OF ETHONOLIC EXTRACT OF *TYLOPHORA INDICA* IN ALLOXAN INDUCED DIABETIC RATS

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ABSTRACT

This study was undertaken to investigate the effect of methonolic extract of *Tylophora indica* (Asclepiadaceae) leafs on blood glucose levels and other biochemical parameters in alloxone (120 mg/kg. b.w., *i.p*) induced diabetic rats. The treatment was given at doses of 200,300,400mg/kg. b.w. for 28 days. After the treatment a significant reduction was observed in fasting blood glucose levels in treated diabetic rats. *Tylophora indica* showed significant decrease ($p < 0.001$) in blood glucose levels. Simultaneously, the alteration in lipid metabolism was partially attenuated as evidenced by decreased serum total cholesterol (TC), triglyceride (TG) and low-density lipoprotein cholesterol (LDL) levels and by increased high-density lipoprotein cholesterol (HDL) concentration in diabetic rat ($p < 0.001$). Observations suggested considerable lowering of lipid profile, bilirubin, and creatinine levels in treated diabetic rats that are close to normal levels after the treatment with *Tylophora indica* in dose related manner. These results suggest that *Tylophora indica* possesses anti diabetic effects in alloxone induced diabetic rats

Keywords: Anti diabetic activity, *Tylophora indica*, Lipid profile, Oral glucose tolerance test.

INTRODUCTION

Diabetes mellitus is a metabolic disorder affecting carbohydrate, fat and protein metabolism. Diabetes mellitus is a chronic disorder of metabolism caused by an absolute or relative lack of insulin. It is characterized by hyperglycemia in the postprandial and/or fasting state, and in its severe form is accompanied by ketosis and protein wasting⁽¹⁾ Besides drugs classically used for the treatment of diabetes (insulin, sulphonylureas, biguanides and thiazolidinediones), several species of plants have been described in the scientific and popular literature as having a hypoglycemic activity.⁽²⁻³⁾ Because of their perceived effectiveness, minimal side effects in clinical experience and relatively low costs, herbal drugs are prescribed widely even when their biologically active compounds are unknown⁽⁴⁾

Tylophora indica Burn. F (Hindi-Antamul, Telugu -Veripala) belongs to the family Asclepiadaceae and has been extensively used in Indian indigenous medicine. The leaves of this plant are the best indigenous substitute for Ipecacuanha. The leaves are traditionally used as an expectorant, emetic, anti-dysenteric⁽⁵⁾ and to treat bronchial asthma and allergic rhinitis.⁽⁶⁾ *T. indica* leaves contain two major alkaloids (Tylophorine, Tylophorinine), three minor alkaloids⁽⁷⁾, tannin, resin, glucose, ceryl alcohol, α -amyrin, quercetin, a flavones identified as kaempferol⁽⁸⁾ and essential oils.⁽⁹⁾ The leaves also contain Na, K, Cu, Zn, Ni, Cd, V, Mo, and Ti⁽¹⁰⁾

Experimentally, extract of *T. indica* produced non-specific anti-spasmodic action on isolated tissues and a fall of blood pressure and prolonged relief in bronchial asthma.⁽¹¹⁾ Pharmacologically it is proved that the alkaloid Tylophorine isolated from *T. indica* produces depression of the central nervous system (CNS).⁽¹²⁾

MATERIALS AND METHODS

Plant material

The methonolic extract of *Tylophora indica* standard dry extract (Batch No 6979) was purchased at AMSAR PRIVATE Ltd. INDORE, Madhya Pradesh

Toxicity studies

Acute oral toxicity (AOT) of ALLT was determined using nulliparous, non-pregnant female mice. The animals were subjected to fasting for 3 hours prior to the experiment and were administered with single dose of the extract dissolved in 2% W\V Tween 80 and observed for mortality for 48 hours (short-term toxicity). Based on the short-term toxicity, the dose administered to the next animal was

determined as per OECD guideline 425⁽¹³⁾. All the animals were also observed for long-term toxicity (14 days). The LD₅₀ of the test extracts were calculated using 'AOT 425' software provided by Environmental Protection Agency, USA.

Drugs

Alloxone monohydrate was purchased from sigma chemicals (St. Louis, U.S.A). All other chemicals used for this study were analytical grade.

Animals

Wister Albino Rats (150–200g) were obtained from the Animal House (743/abc), Prist University, Thanjavuru. Rats were maintained on standard pellet diet and tap water *ad libitum*. They were kept in clean cages under a 12 hour light/dark cycle and room temperature 22–24°C and were acclimatized to the environment for 2 weeks prior to experimental use. This study was conducted according to the guidelines approved by the Institutional Animal Ethics Committee.

Induction of diabetes

Diabetes was induced by injection of a single intra-peritoneal dose of Alloxan monohydrate (freshly prepared in 0.1% normal saline). Overnight fasted rats were injected with Alloxan (alloxan; 120 mg/kg body wt., *i.p*) to induce diabetes. Diabetes was confirmed by glucose estimation. Animals with plasma glucose level > 200 mg / dl were selected for the study. Diabetic induced Animals were grouped for further study. After 3 days of alloxan induction, treatment was started.

Experimental Design

In this experiment a total number of 48 rats were used. The rats were divided in to seven groups each having six animals. Group 1 consisted of normal rats treated with vehicle (normal saline) which served as control, Group 2 contained Alloxan (120mg/kg) treated rats that served as diseased control rats, Group 3 Glibenclamide (50mg/kg) treated rats, Group 4, 5 and 6 groups were diabetic rats treated with ethanolic extract of *Tylophora indica* at doses of 200,300 and 400 mg/kg respectively according to the body weight for a period of 28 days. All the doses were administered orally.

Determination of Glucose in Serum⁽¹⁴⁾

The serum glucose level was estimated in overnight fasted controls, diseased controls (DC) and drug treated diabetic animals at a dosage of 200, 300 and 400mg/kg, body weight. Blood was collected in vials from the retro orbital plexus with capillary tube and was allowed to clot to separate serum. It is then centrifuged at 2500 rpm for 10 min to

obtain clear serum. 10 µl of serum was added with 1 ml of kit reagent incubated at 37 °C for 10 minutes. Mix and read absorbance at 505 nm.

Oral glucose tolerance test ⁽¹⁵⁾

Treated and alloxan induced diabetic rats, fasted overnight but provided with water (ad libitum) were administered test samples orally one and half hour prior to the oral glucose load of 2mg/kg body weight. Glucose concentration was measured before administration and subsequently at 30, 60, 90 and 120 min after the glucose administration. The control group received only the glucose load.

Serum Cholesterol and triglycerides ⁽¹⁶⁾

Cholesterol was measured by a direct colorimetric method.

Determination of kidney parameters ⁽¹⁷⁻²⁰⁾

Estimation of creatinine, bilirubin, total protein and albumin were measured for all groups of treated, normal and diabetic control animals.

Determination of liver parameters

Estimation of SGOT, SGPT and ALP were measured for all groups of treated, normal and diabetic control animals. Diagnostic kits for the estimation of SGOT, SGPT, ALP and serum were purchased from local supplier (Sai chemicals) manufactured by Ranbaxy Diagnostics Ltd., New Delhi, India.

Determination of Erythrocyte glycosylated hemoglobin ⁽²¹⁾

A new colorimetric method was used to estimate the amount of glucose bound to the haemoglobin.

Determination of blood urea nitrogen ⁽²²⁾

Estimation of blood urea nitrogen (BUN) was done by the diacetyl monoxime method by Kanter (1975). Protein free filtrate was prepared. To 0.5mL of protein-free filtrate 3.5mL of distilled water, 0.8mL diacetylmonoxime (2%), and 3.2mL sulfuric acid-phosphoric acid reagent (reagent was prepared by mixing 150mL 85% phosphoric acid with 140mL water and 50mL of concentrated sulphuric acid) were added. The reaction mixture was placed in a boiling water bath for 30 min and then cooled. Absorbance was recorded at 480 nm.

Statistical Analysis

Results are expressed as mean ± S.E.M. The difference was compared using two way ANOVA followed by Bonferroni-post test. $p < 0.001$ was considered as significant.

RESULTS

Effect on Glucose in Serum

Table 1: The effect of repeated oral administration of ETI in normal and alloxone rats is shown in Table.1. In normal rats, fasting plasma

glucose levels were practically similar at 0, 7, 14, 21, and 28 days after distilled water administration. However, ETI (200,300 and 400mg/kg) decreased plasma levels to 60.9%, 63.55% and 68.5% in dose dependent manner. The standard drug glibenclamide showed 71% reduction in glucose levels when compared with disease control group.

Effect on oral glucose tolerance test

Table.2, fig.1 shows the effect of different concentrations of ETI on OGT at significant level ($P < 0.001$). There is an increased blood glucose level in all treated animals at 60 min as compared to before treatment 35%, 37% and 38% respectively for 400,300 and 200mg/kg, body weight. There is a considerable decrease in elevated blood glucose concentration at 120 min in order of 30%, 29% and 29%. (ETI 200, 300 and 400 mg/kg). Glibenclamide also improve the glucose tolerance test up to 2 hrs.

Effect on Serum Cholesterol and triglycerides

Table. 3, shows the effect of ETI on serum lipid concentration as studied after extract administration for 4 weeks in rats. There were significant differences in most of the lipid profiles between the ETI treated rats and disease control group (Table 3).

Serum concentration of triglyceride in the extract (200, 300 and 400 mg/kg) significantly decreased by 56%, 57% and 59% and there was dose dependent decrease in LDL levels 62%, 68% and 75% and Glibenclamide 78%. But the level of HDL-cholesterol significantly increased 46%, 41% and 36%. Glibenclamide increased by 32% significantly at $p < 0.001$.

Effect on kidney parameters

Table.4, shows the effect of ETI (200, 300 and 400 mg/kg) and glibenclamide decreased creatinine levels in dose dependent manner by 45%, 53%, 62% and 71%. Bilirubin levels by 46%, 49%, 52% and 62%. Albumin levels by 53%, 55%, 60% and 64% respectively (Fig 3).

Effect on biological parameters

Table 5, indicates, there is a significant decreased effect on SGOT, SGPT and ALP at $p < 0.001$ as compared to the alloxan induced rats at near to the standard drug glibenclamide with *Tylophora indica*. it shows dose dependent effect on biological parameters.

Effect on Erythrocyte glycosylated haemoglobin and BUN

Table 6: HbA1c levels are decreased by 64% and 57%, 58%, 59% for glibenclamide and ETI (200, 300 and 300 mg/kg) as shown in Table 4 in dose dependent manner. Also there is reduction of BUN by ETI 38%, 39% and 40% (200,300 and 400mg/kg) at significant level $P < 0.001$ as compared to alloxone treated group as shown with Glibenclamide.

Table 1: Effect of ETI on Serum Glucose levels in alloxan induced diabetic rats

Treatment (mg/kg)	0 DAY	7 th DAY	14 th DAY	21 st DAY	28 th DAY
Normal	112.66±0.7	96.16±1.19 ^a	95.66±1.20 ^a	101.33±1.9 ^a	96.83±0.79 ^a
D.control	307±3.93	319±4.50	324.33±5.4	311.81±5.1	302.83±6.1
Standard	315.66±6.5	101.83±2.1 ^a	96±1.18 ^a	90.83±0.88 ^a	85.50±0.99 ^a
ETI-200mg/kg	294.16±0.9	166.50±2.2 ^a	145.5±1.11 ^a	132.66±0.8 ^a	118.50±1.3 ^a
ETI-300mg/kg	304.16±1.6	137.83±1.4 ^a	126.16±0.6 ^a	120.66±0.7 ^a	110.66±2.3 ^a
ETI-400mg/kg	301.88±169	115.83±1.2 ^a	105.16±0.7 ^a	103.33±0.4 ^a	95.66±0.66 ^a

Results are expressed as mean±SEM, n=6, ^a $p < 0.001$, ^b $p < 0.01$, ^c $p < 0.05$, vs alloxan induced group using Two way ANOVA followed by Bonferroni-post test.

Normal: distilled water

Disease control: alloxane (120mg/kg,b,w)

Standard: glibenclamide (50mg/kg)

ETI: ethanolic extract of tylophora indica

Table 2: Effect of ETI on Oral glucose tolerance test in alloxan induced diabetic rats

Treatment(mg/kg)	0(min)	30(min)	60(min)	90(min)	120(min)
normal	124.3±2.24	257±6.03	295.8±5.6	123.8±6.84	89.3±4.4
D.control	367.7± 0.4	431.3± 0.6	533.5±0.4	425.5 ±0.3	383.1± 0.4
standard	275.5± 0.8	296.3± 0.6	320.5± 0.42	283±0.71	265.6± 0.3
ETI-200mg/kg	291.1± 0.3	312± 0.2	342 ±0.25	290.5 ±0.2	274.6± 0.2
ETI-300mg/kg	242.9± 4.2	306.8 ±0.4	335.3 ±0.6	274.3± 0.4	230.5 ±0.4
ETI-400mg/kg	279± 0.02	302.1± 0.4	326± 0.36	282.5 ±0.9	266.1 ±0.3

Results are expressed as mean±SEM,n=6,p<0.001, ^b p<0.01, ^c p<0.05 vs alloxan induced group using Twoway ANOVA followed by Bonferroni-post test.

Normal: distilled water

Disease control: alloxane (120mg/kg,b,w)

Standard: glibenclamide (50mg/kg)

ETI: ethanolic extract of tylophora indica

Table 3: Effect of ETI on Lipid profile in alloxan induced diabetic rats

Treatment(mg/kg)	TG(mg/dl)	TC(mg/dl)	HDL(mg/dl)	LDL(mg/dl)	VLDL(mg/dl)
normal	154±1.527 ^a	126±1.36 ^a	64.66±1.33 ^a	32.6 ±0.49 ^a	30.86±0.329 ^a
D.control	355.3±1.78	252±1.23	34.2±0.541	145.5 ±0.61	71.06±0.35
Standard	137.4± 1.18 ^a	122 ±0.73 ^a	66± 0.36 ^a	31.3± 0.5 ^a	25.3± 0.23 ^a
ETI200mg/kg	155.6±0.84 ^a	134±0.74 ^a	49.8 ±0.42 ^a	54.5 ±0.42 ^a	31.13±0.16 ^a
ETI300mg/kg	152.5±0.99 ^a	129.8±0.73 ^a	56.3± 0.42 ^a	46 ±0.36 ^a	30.46±0.204 ^a
ETI400mg/kg	144.8±1.77 ^a	124.6±0.76 ^a	61.5± 0.42 ^a	36± 0.25 ^a	28.96±0.035 ^a

Results are expressed as mean±SEM,n=6,p<0.001, ^b p<0.01, ^c p<0.05 vs alloxan induced group using Two way ANOVA followed by Bonferroni-post test.

Normal: distilled water

Disease control: alloxane(120mg/kg,b,w)

Standard: glibenclamide(50mg/kg)

ETI: ethanolic extract of tylophora indica

Table 4: Effect of ETI on Different parameters in alloxan induced diabetic rats:

Treatment(mg/dl)	HbA _{1c} %	BUN(mg/dl)
normal	3.13±0.18 ^a	21.61±0.42 ^a
D.control	8.20±0.18	48.03±0.33
Standard	2.81±0.15 ^a	20.96±0.27 ^a
ETI200mg/kg	3.39±0.14 ^a	27.55±0.36 ^a
ETI300mg/kg	3.31±0.14 ^a	25.58±0.32 ^a
ETI400mg/kg	3.26±0.06 ^a	24.61±0.39 ^a

Results are expressed as mean±SEM,n=6,p<0.001,^b p<0.01, ^c p<0.05 vs alloxan induced group using Two way ANOVA followed by Bonferroni-post test.

Normal: distilled water

Disease control: alloxan (120mg/kg,b,w)

Standard: glibenclamide (50mg/kg)

ETI: ethanolic extract of tylophora indica

Table 5: Effect of ETI on Different parameters in alloxan induced diabetic rats

Treatment(mg/dl)	SGOT(IU/L)	SGPT(IU/L)	ALP(IU/L)
Control	168.50±0.24 ^a	76.86±0.22 ^a	152.75±0.23 ^a
D.control	179.18±0.28	83.58±0.18	186.55±0.50
Standard	170.13±0.14 ^a	77.60±0.29 ^a	153.40±0.18 ^a
ETI-200mg/kg	174.83±0.91 ^a	79.50±0.76 ^a	156.15±0.20 ^a
ETI-300mg/kg	169.68±1.00 ^a	77.08±0.26 ^a	154.54±0.60 ^a
ETI-400mg/kg	168.05±0.15 ^a	76.66±0.33 ^a	153.18±0.32 ^a

Results are expressed as mean±SEM, n=6, p<0.001, ^b p<0.01, ^c p<0.05 vs alloxan induced group using Two way ANOVA followed by Bonferroni-post test.

Normal: distilled water

Disease control: alloxan (120mg/kg,b,w)

Standard: glibenclamide (50mg/kg)

ETI: ethanolic extract of tylophora indica

Table 6: Effect of ETI on Different kidney parameters in alloxan induced diabetic rat

Treatment (mg/dl)	Creatinine (mg/dl)	T.bilirubin (mg/dl)	D.bilirubin (mg/dl)	ID.bilirubin (mg/dl)	T.P (mg/dl)	Albumin (mg/dl)
Normal	0.685±0.01 ^a	0.98±0.06 ^a	0.75±0.06 ^a	0.23±0.41 ^a	7.5±0.096 ^a	4.05±0.06 ^a
D.control	1.812 ±0.02	1.8±0.045	1.25±0.074	0.58±0.05	13.45±0.143	9.48± 0.13
Standard	0.54± 0.01 ^a	0.68 ±0.03 ^a	0.63± 0.02 ^a	0.15 ±0.02 ^a	6.3± 0.03 ^a	3.46 ±0.07 ^a
ETI200mg/kg	0.983±0.08 ^a	0.96±0.04 ^a	0.75±0.047 ^a	0.216±0.03 ^a	7.216±0.07 ^a	4.48±0.06 ^a
ETI300mg/kg	0.83±0.02 ^a	0.916±0.06 ^a	0.733±0.05 ^a	0.233±0.02 ^a	7.16±0.07 ^a	4.26±0.05 ^a
ETI400mg/kg	0.673±0.05 ^a	0.866±0.03 ^a	0.7±0.023 ^a	0.16±0.021 ^a	6.43±0.076 ^a	3.85±0.042 ^a

Results are expressed as mean±SEM,n=6,p<0.001^b p<0.01, ^c p<0.05 vs alloxan induced group using Two way ANOVA followed by Bonferroni-post test.

Normal: distilled water

Disease control: alloxane (120mg/kg,b.w)

Standard: glibenclamide (50mg/kg)

ET : ethanolic extract of *tylophora indica*

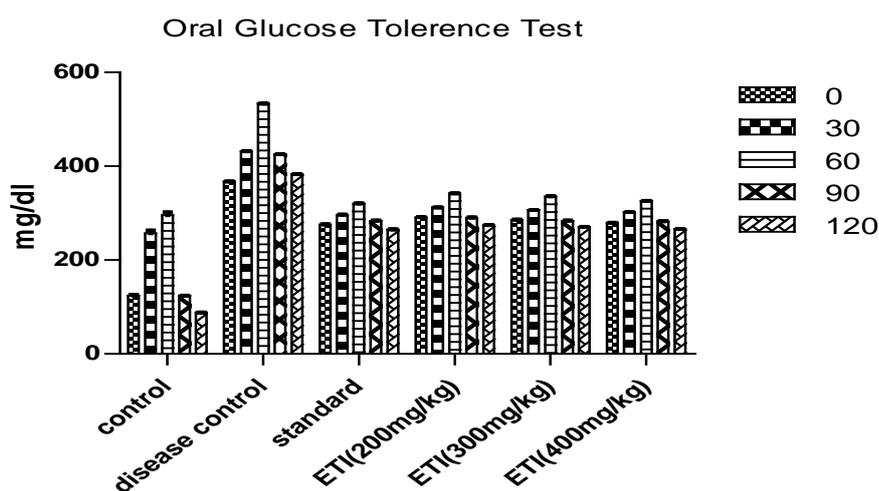


Fig. 1: Effect of ETI on Oral glucose tolerance test in alloxan induced diabetic rats

Results are expressed as mean±SEM,n=6,p<0.001, ^b p<0.01, ^c p<0.05 vs alloxan induced group using Two way ANOVA followed by Bonferroni-post test.

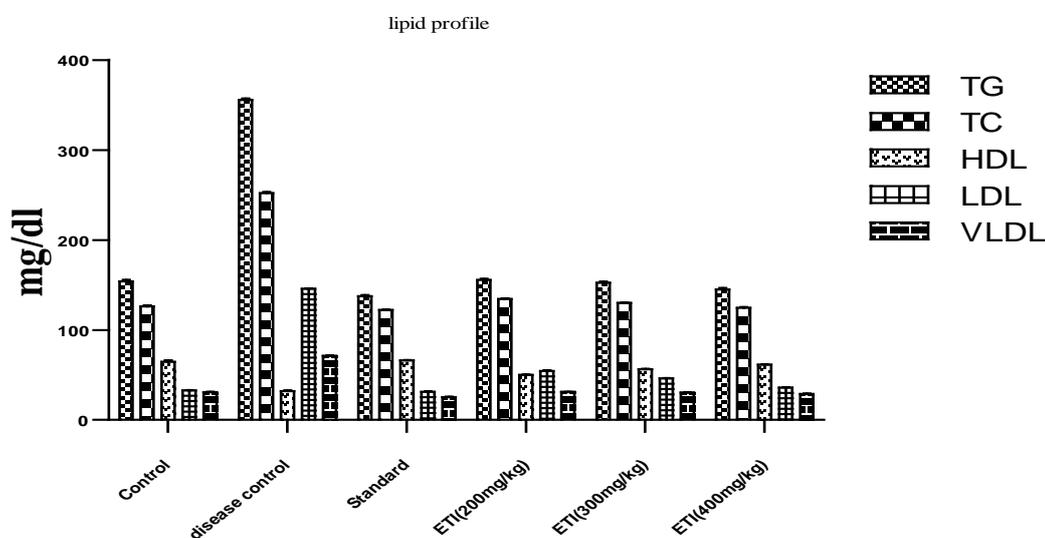


Fig. 2: Effect of ETI on Lipid profile in alloxan induced diabetic rats

Results are expressed as mean±SEM,n=6,p<0.001, ^b p<0.01, ^c p<0.05 vs alloxan induced group using Two way ANOVA followed by Bonferroni-post test.

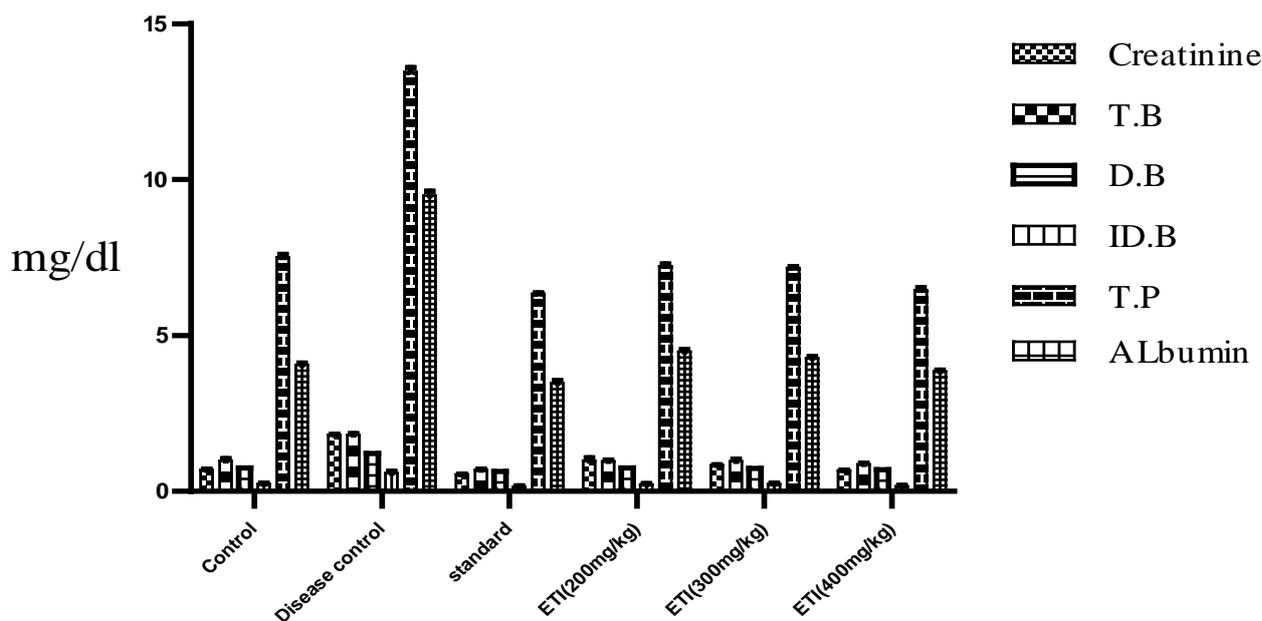


Fig. 3: Effect of ETI on difernt parameters in kidney in alloxan induced diabetic rats

Results are expressed as mean±SEM, n=6, $p < 0.001$, ^b $p < 0.01$, ^c $p < 0.05$ vs alloxan induced group using Two way ANOVA followed by Bonferroni-post test

DISCUSSION

Alloxan has been observed to cause a massive reduction in the number of the β -cells of the islets of Langerhans and induced hyperglycemia (23). In our approach, we demonstrated the efficacy of alloxan through the glibenclamide studies in diabetic rats as well as in normal hyperglycemic rats. In the present study, the hypoglycemic activity of crude extract from *Tylophora indica* leaves was evaluated in control, disease control and alloxan-induced diabetic rats. A single oral administration with the crude extract of *Tylophora indica* caused a significant decrease in serum glucose levels in all rat groups. Moreover, these doses of the crude extract produced a significant time-dependent hypoglycemic effect as shown throughout the period studied. These results in glucose-fed hyperglycemic normal rats reinforce the hypothesis that the hypoglycemic mechanism involves an insulin-like effect, probably, through peripheral glucose consumption (24-26). Although the crude extract of *Tylophora indica* leaves displayed a significant hypoglycemic effect in normal rats, the main mechanism by which *Tylophora indica* brings about its hypoglycemic action probably is by stimulating peripheral glucose consumption. In this context a number of other plants have also been reported to have hypoglycemic effects (25, 27, and 28). To our knowledge, this is the first study demonstrating an anti-hyperglycemic effect of *Tylophora indica* in diabetic animals.

The effect of glibenclamide on glucose tolerance has been attributed to enhanced activity of β -cells of the pancreas resulting in secretion of larger amounts of insulin (29). In contrast to the oral anti diabetic agents, the exogenous administration of insulin is well known to produce hypoglycemia in both normal and alloxan induced subjects (30). In our model, this action was confirmed. It is, therefore, conceivable that the hypoglycemic principles in the fractions of *Tylophora indica* plant exert a direct effect on diabetic rats. In induced-diabetic rats, neither crude extract nor fractions can act directly by stimulating the release of insulin since alloxan treatment causes permanent destruction of β -cells. Different mechanisms of action to reduce blood glucose levels with the help of plant extracts already exist. Some plants exhibit properties similar to the well-known sulfonyleurea drugs like glibenclamide; they reduce blood

glucose in normoglycaemic animals (31-32). Some other plants act like biguanides such as metformin which is an anti hyperglycaemic compound; they do not affect blood glucose in normal state (33-35). We hypothesized that *Tylophora indica* could have a sulfonyleurea-like mechanism since it decreased blood glucose in normoglycaemic rats such as glibenclamide. Sulfonyleurea compounds lower blood glucose in normal and type 2 diabetic animals by stimulating insulin release from β -pancreatic cells. It is also known that alloxan selectively destroys insulin-secreting β -cells in the Islets of Langerhans and their effects are irreversible (36). In the present study, the dose of alloxan (120 mg/kg, i.p) was selected in order to partially destroy the pancreatic β -cells. In these conditions, insulin was secreted but not sufficiently to regulate the blood glucose.

Generally, diabetic models are used to identify a successful treatment for hypercholesterolemia. The most common lipid abnormalities in diabetes mellitus are changes in the plasma cholesterol and triglyceride concentration (37), which certainly contributes to the development of vascular disease. Our results showed a significant decrease ($p < 0.001$) in total cholesterol levels in diabetic groups treated with ETI (200, 300 and 400mg/kg). Administration of all diets for 4 weeks exhibited significant decrease ($p < 0.001$) in triglyceride level of hyperglycemic groups. However, there was no significant difference in reduction of triglyceride level at week 4 between treated and control rats. High levels of total cholesterol and LDL-cholesterol are major coronary risk factors. Results showed a significant decrease ($p < 0.001$) in LDL-c level in diabetic rats fed with ETI (200,300 and 400mg/kg) compared to disease control. (38) Reported that most of the treatments of anti-hypercholesterolemia agents could decrease both LDL and HDL cholesterol levels. Interestingly, our study found that HDL-c levels had increased significantly ($p < 0.001$) in diabetic rats treated with ETI. These results indicated that *Tylophora indica* extract might have some protective effects against hypercholesterolemia risks in diabetes.

The raise in the levels of serum bilirubin is most sensitive and confirms the intensity of jaundice (40-43). It was reported that the increase in plasma bilirubin (hyper-bilirubinemia) may be resulted from the decrease of liver uptake, conjugation or increased bilirubin

production from hemolysis and this finding coincided with the decrease in total erythrocyte counts. Also, the elevation in plasma bilirubin indicates liver damage as confirmed by the changes in the activities of liver enzymes. Furthermore, the improvement of the liver damage by oral administration of ethanolic extract of *Tylophora indica* (200,300 and 400mg/kg) could be confirmed by studying their effect on the level of plasma bilirubin. However, Ethanolic extract of *Tylophora indica* intake produced significant ($p < 0.001$) decrease in plasma bilirubin as compared to the diabetic rats. It is an indication that *ETI* leaves have liver protective response.

Blood albumin levels are selective markers of liver injury that rodents such as rats and mice exhibit⁽⁴²⁻⁴³⁾. Blood albumin levels in the diabetic group; however, were many folds higher than normal controls. Treatment with ethanolic extract of *Tylophora indica* was seen in lipidemic treated controls. The observations of the present study indicate that the extracts of *Tylophora indica* protect liver disorder effects of hyperlipidemia.

Total protein is useful for measuring gross changes in protein levels caused by various disease states. Albumin is quantitatively the major single contributor to the total protein. Measurement of total protein levels alone may be misleading and may be normal in view of the quite marked additional information. Increased protein levels were observed in dehydration and diarrhea. However, ethanolic extract of *Tylophora indica* intake produced significant ($p < 0.001$) decrease in plasma total protein levels when compared to the hyperlipidemic rats. It is an indication that *ETI* fruit has liver protective response.

The significant of SGPT, an enzyme found primarily in liver and heart is far greater enhanced and released into the blood stream is the result of liver abnormality. If therefore serve as a fairly specific indicator of liver status and its elevated levels in serum indicates liver damage. *Tylophora indica* reduces the SGPT levels when compared to the diseased group indicating its protective effect over liver and heart complications.

SGOT is an enzyme found primarily in the cells of the liver, heart, skeletal muscles, kidneys, and pancreas and to a lesser extent in red blood cells. Its serum concentration is in proportional to the amount of cellular leakage or damage. It is released into serum in larger quantities when any one of these tissue is damaged. Its increased levels are usually associated with liver disease or heart attacks. *Tylophora indica* decreased the SGOT level significantly when compared to the other diseased groups, other groups were not reduced the SGOT levels when compared to the control group. This is an indication of the protective effect on liver and heart.

Glycosylated hemoglobin reflects the mean blood glucose concentration. Hence HbA_{1c} is now considered as the most reliable marker of glycemic control in diabetes mellitus⁽⁴⁴⁾. In diabetes, high blood glucose reacts with hemoglobin and thus increased glycosylated hemoglobin is formed. We observed decreased hemoglobin and increased glycosylated hemoglobin levels in diabetic rats. Increased hemoglobin in *ETI extract* treated diabetic rats indicated decreased blood glucose level and glycosylated hemoglobin. Blood urea nitrogen, after nickel treatment reflects its interaction with the cell membrane, leading to altered cell membrane permeability and increased enzyme leakage. This was reduced significantly after *Tylophora* treatment and pretreatment, thus showing its efficacy in enhancing kidney function.

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