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EVALUATION OF DIABETIC COMPLICATIONS, NEURO, HEPATO, CARDIO AND NEPHRO PROTECTIVE EFFECTS OF ETHANOLIC EXTRACT OF THE WHOLE PLANT OF *TAXILLUS TOMENTOSUS* IN ALLOXAN INDUCED DIABETIC RATS

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ABSTRACT

Diabetes mellitus (DM) is a world's fastest growing metabolic disease with high prevalence worldwide; associated with markedly increased morbidity and mortality rate and reduced the quality of life. It is the third commonest disease in the world affecting approximately 2.8% of global population. Persons with diabetes are at increased risk for macro vascular and micro vascular disease. Currently available synthetic antidiabetic agents produce serious side effects. This leads to a demand for herbal products with antidiabetic activity and fewer side effects. Hence the effects of oral administration of ethanolic extract of Taxillus tomentosus has been studied in alloxan induced diabetic Wister albino rats. The diabetic rats treated with ethanolic extract of whole plant of Taxillus tomentosus Tiegh with low dose and high dose, Metformin orally as an anti-hyperglycemic standard for 14 days. Test extract exhibited a significant dose dependent anti hyperglycaemic activity compared to diabetic control which is less potent than reference standard metformin. The results indicate that the ethanolic extract of Taxillus tomentosus is endowed with antidiabetic activity.

KEYWORDS: Taxillus tomentosus, Hyperglycemia, Alloxan, Diabetic complications, allodynia.

1. INTRODUCTION

1.1 Introduction to Diabetes mellitus: Diabetes mellitus is a group of chronic progressive metabolic disorder of multiple aetiology characterized by hyperglycemia with disturbances in carbohydrate, fat and protein metabolism along with specific long-term complications affecting the different organ of the body which arises from complex interactions between multiple genetic and environmental or lifestyle factors.

This chronic disease is characterized by the presence of hyperglycemia due absolute or relative deficiency of insulin or a defect in the action of insulin or both.

Diabetes has traditionally been divided into insulin-dependent (type 1) accounts for 5–10% of all diagnosed cases, and non-insulin-dependent (type 2) diabetes accounts for 85–90% of patients with DM.

The importance of protecting the body from hyperglycemia cannot be overstated; the direct and indirect effects on the human vascular tree are the major source of morbidity and mortality in both type 1 and type 2diabetes. Generally, the injurious effects of hyperglycemia are separated into macro vascular complications like coronary artery disease (CAD), peripheral vascular disease (PVD), and stroke and micro vascular complications like diabetic neuropathy, and retinopathy, peripheral and autonomic neuropathies, and lower extremity disease. It is important for physicians to understand the relationship between diabetes and vascular disease; Micro vascular complications are the major risk in type 1 diabetes, while macro vascular complications are the major cause of morbidity and mortality in type 2 diabetes. The major aim of diabetes management is to prevent secondary complications.

Diabetes mellitus (DM), long considered a disease of minor significance to world health, is now taking its place as one of the main threats to human health in the 21st century. It is the most common non-communicable disease worldwide and the fourth to fifth leading cause of death in high income countries and still growing to an alarming level. The global figure of people with diabetes is set to rise from the current estimate of 150 million to 220 million in 2010 and 300 million in 2025.

According to the World Health Organization (WHO) estimates, India had 32 million diabetic subjects in the year 2000 and this number would increase to 80 million by the year 2030.

Type 2 diabetes mellitus is increasingly prevalent worldwide, conferring major burdens on health and health care costs. Type 2 diabetes may be largely preventable.

Modern medical diabetic care uses a huge range of lifestyle management and pharmaceutical interventions aimed at preventing and controlling hyperglycemia. In addition to ensuring the adequate delivery of glucose to the tissues of the body, treatment of diabetes attempts to decrease the likelihood that the tissues of the body are harmed by hyperglycemia.

The number of people with diabetes is increasing due to population growth, aging, urbanization, and increasing prevalence of obesity and physical inactivity.

1.2 Introduction to Taxillus tomentosus

Taxillus tomentosus is a parasitic shrub with dark brown branches, Active, shrub, Dicot, perennial Branches lenticellate, young shoots fulvous tomentose. Leaf stalks are 5-7 cm long, rusty-hairy. Leaves are hairless; ovate-oblong to elliptical, size is about 5 x 2.5 cm, obtuse, glabrous above; nerves 4-5 pairs; petiole 5 mm long. Flowers are green, with 1 cm long green flower-tube, and narrow green petals, 4 mm long. Flowers are 3-8 in number, fascicled; pedicels 2 mm long; calyx 2 mm long, lobes triangular; corolla greenish white, pink at base, to 16 mm long, densely hairy, tube gibbous, lobes spreading; stigma hemispheric. Berry, sub-globose, tomentose. Bracts are prominently longer than the sepal tube, an identifying feature, Flower and fruit from December-January.

Taxillus tomentosus was taken from the Greek words taxis (regular) and illus (diminutive, small) toh-men-toh-sus -- covered with fine, matted hairs it is commonly known as: hairy mistletoe, Loranthus tomentosus.

Taxillus tomentosus is one of the herbal plant belongs to family loranthecaea and the genus Taxillus, it is found in Found in Maharashtra (Sindhudurg), Karnataka (Chikmagalur, Coorg, Hassan, Mysore, S. Kanara) Kerala (Idukki, Kannur, Malapuram, Palakkad, Pathanamthitta, Thiruvananthapuram, Thrissur) Tamil Nadu (Coimbatore, Dindigul, Kanniyakumari, Nilgiri, Tirunelveli) it is also found in srilanka. Literature review reveals that this plant has no proven activity.

Fig.: Plant of Taxillus tomentosus.

2. MATERIAL AND METHODS

2.1 Plant material: Plant material: The plant of Taxillus tomentosus plants were collected from the certified ayurvedic wholesaler. The plant was identified and authenticated by Asst. Prof. K. Dr. K. Madhava chatty, MSc, Med, Department of Botany, S.V. University, Tirupati.

2.2 Preparation of Taxillus tomentosus extract: The collected fresh whole plant materials were dried in shade (2 days) and then dried in a hot air oven at 25ºC for three days and they were made in to coarse powder with the use of mixer grinder. The powdered drug then was passed through sieves no. #44 and powdered drug obtained is used for extraction process. The powder of Taxillus tomentosus obtained were weighed separately and Approximately 250 gm. of powder was transferred to a round bottomed flask and then kept to continuous heat extraction with Soxhlet apparatus using 95% ethanol (prepared by using 95 parts of ethanol and 5 parts of distilled water) for 72 hours. Then the extract of ethanol was concentrated by placing it aside for some time. Extract obtained was dried by placing it on a big petri plate on electric water bath (70ºC) and then kept in an oven at 30ºC for 2 hour. The extract obtained was kept for drying and stored in vacuum desiccators. The percentage yield of the extract was 11.98%.

2.3 Materials used in this study are as follows

2.3.1: Chemicals: Distilled water, Alloxan monohydrate, (Sigma Aldrich, USA), Metformin (Alembic Pharma) Chloroform (Fisher scientific), Diethyl ether (Fisher scientific) Ethanol (Fisher scientific) Tween-80 (as emulsifier to prepare the suspension of plant extract) and all other chemicals were used AR grade and ethanolic extract of Taxillus tomentosus (ETT1 and ETT2).

2.3.2: Experimental Animal: The Wister albino rats of either sex (200-250g) were obtained from the central animal house of Sigma institute of clinical research & administration Pvt. Ltd, Hyderabad. Animals were housed at a temperature of 24±2°C and relative humidity of 30-70%. A 12:12 hrs. Light: dark cycle was followed. All animals had free access to water and standard pellet laboratory animal diet. Animals were acclimatized to laboratory conditions before the experiment procedure used in this study were reviewed. Institutional Animal Ethics Committee (769/2010/CPCSEA) approved the study protocol.

2.3.3: Equipment: Borosilicate Soxhlet extractor, Round bottomed flask, Sieves of different sizes especially #44, Sieve shaker, Refrigerator, Biochemical Auto analyzer (Robonik), Robonik diagnostic kits (creatinine, SGOT, SGPT, urea, uric acid, cholesterol, glucose, ALP, Triglycerides, HDL) Centrifuge (Biofuse Pico), electronic digital weighing (Apex), EDDY's Hot plate analgesometer MK-111 (Sisco), Glucose check monitoring system (Aspen diagnostics), micro pipette, disposable syringes.

2.4 Phytochemical screening of Taxillus tomentosus: Phytochemical screening of plant extract shows the presence of carbohydrate, protein, flavonoids, alkaloids, steroids and saponins.

2.5 Toxicity studies of Taxillus tomentosus: Toxicity study involve a test in which a single dose of drug extract is used in each animal on occasion only for determination of LD_{50} or median lethal dose i.e. the dose which kills 50% of animals of a particular species. LD_{50} values are determined for 15 days of study. Ethanolic extract of drug is used to determined LD_{50} values.

Acute toxicity studies were performed according to OECD-423(2001) guidelines category IV substance (acute toxic class method). The doses were selected 5mg/kg, 50mg/kg, 300mg/kg and 2000mg/kg.

For this purpose Swiss albino rats were employed. They were non pregnant and nulliparous and there weight lies between 200-250gms.

Albino rats (n=3) of either sex selected by random sampling technique. The animals were fasted for 4 hrs. with free access to water only. The plant extracts of Taxillus tomentosus were administered orally with maximum dose of 2000 mg/kg body weight. The mortality was observed for three days. If mortality was observed in 2/3 or 3/3 of animals, then the dose

administered was considered as a toxic dose. However, if the mortality was observed only one mouse out of three animals then the same dose was repeated again to confirm the toxic effect. If mortality was not observed, the procedure was then repeated with higher dose.

The plant extracts of Taxillus tomentosus didn't shown any mortality and toxicity even at highest dose of 2000 mg/kg body weight employed. So, the extracts were safe for long term administration.

3. METHOD

3.1 Selection of Dose of Taxillus tomentosus: In the present study we have selected two doses i.e. 200 mg/kg and 400 mg/kg of body weight for evaluating antidiabetic and activity against diabetic complications, the selection of doses is based upon toxicity study. In the toxicity studies up to 2000 mg/kg body weight animals have not shown any signs of toxicity, morbidity and mortality. So 10% of the maximum dose has been chosen i.e. 200 mg/kg body weight, the two doses were selected one as submax (200 mg/kg of body weight) and other is supramax (400 mg/kg body weight).

3.2 Preparation of the drug solution

3.2.1 Taxillus tomentosus: The obtained dried Ethanolic extract of Taxillus tomentosus was given orally in form of suspension which was prepared by using distilled water as vehicle and Tween-80 (emulsifier).

3.2.2 Metformin: Solution was prepared by dissolving Metformin (Alembic) in distilled water. The drug was prepared daily and was stored at room temperature away from sunlight and moisture. The volume of drug solution was calculated based upon the body weight of the animal.

3.2.3 Alloxan monohydrate: Alloxan monohydrate 5% solution, dissolved in normal saline was used in this study at the dose of 150mg/kg to induce diabetes in rats.

3.3 Pharmacological screening for antidiabetic activity

3.3.1 Experimental Design: - Alloxan induce diabetic model

The chemical method was employed here to study the antihyperglycemic activity. Alloxan monohydrate was used to induce diabetes. Fifteen adult Wistar albino strain rats of either sex weighing 200-250g selected and they were randomly divided into five groups. Each group consisted of 3 animals. The treatment period was considered for 14 days. Animals were fed

with pellet diet and water throughout the experiment. Animals were acclimatized to laboratory conditions before carrying out any experimental work.

Group-1: Diabetic control, 3 diabetic rats were used as control which were remain untreatable for 14 days.

Group-2: 3 rats are grouped as normal which is used to compare the test drug which was fed 0.5 ml of 5% Tween-80 in distilled Water orally for 14 days.

Group-3: 3 Diabetic rats were used as standard that were treated orally with Metformin 14.2mg/kg for 14 days.

Group-4: 3 Diabetic rats were used as test group which was treated orally with lower dose 200 mg/kg of ethanolic extract of Taxillus tomentosus in form of suspension for 14 days.

Group-5: 3 Diabetic rats were used as test group which was treated orally with higher dose 400 mg/kg of etahnolic extract of Taxillus tomentosus in form of suspension for 14 days.

3.4 Assessment of anti-diabetic activity

3.4.1 Evaluation of blood glucose levels and body weight

Blood glucose levels were measured with a portable glucometer (CONTOUR TS) on 0^{th}, 1^{st}, 7^{th} and 14^{th} day. In brief, blood was withdrawn from the rats using tail vein rupture method, and a drop of blood was placed on the glucometer strip loaded in the glucometer for blood glucose determination. During the experiment, blood glucose levels and body weights were verified in the interim of each week.

3.4.2 Evaluation of nociception (neuroparameter)

1. **Tail immersion** (Hot water) test: Tail of rat was immersed in a hot water bath (52.5 ± 0.5 °C) until tail withdrawal (flicking response) or signs of struggle were observed the cut-off time 12 s was taken in order to avoid damage to the tail immersed and it was measured on 1^{st}, 7^{th}, 14^{th} day of study. Shortening of the tail withdrawal time indicates hyperalgesia.

2. Eddy's Hot plate test: The hyperalgesic response on the hot-plate is considered to result from a combination of central and peripheral mechanisms[0]. In this test, animals were individually placed on a hot-plate (Eddy's Hot-Plate) with the temperature adjusted to 55 ± 1 °C. The latency to the first sign of paw licking or jump response to avoid the heat was taken as an index of the pain threshold and it was measured on 1^{st}, 7^{th}, 14^{th} day of study; the cut-off time was 10 s in order to avoid damage to the paw.

3. Assessment of Cold allodynia: Allodynia is hallmark of neuropathic pain, and diabetic neuropathy pain in experimental animals. 2 h after assessment of thermal hyperalgesia cold allodynia was assessed by measuring paw withdrawal latency (PWL). Ice cold water (4±10C) was taken in beaker. The paw of rat was submerged gently in water and the withdrawal time was measured on 1, 7th and 14th day after chronic constriction injury and in the case of diabetic neuropathy it was assessed weekly after confirmation of diabetes. A cut off 20 sec was maintained throughout the experiment.

3.4.3 Evaluation of biochemical parameter

On day 14th, blood was collected by retro orbital puncture under mild ether anesthesia from overnight fasted rats before scarification and fasting blood sugar was estimated. The blood was then subjected to centrifugation to obtain the serum. Serum analyzed for serum creatinine, serum urea, serum uric acid, SGOT, SGPT, ALP, triglycerides, cholesterol and HDL were estimated.

3.4.5 Histopathology: At the end of the study period (on 14th day), animals from all the five groups were anesthetized under mild ether anesthesia and sacrificed; pancreas was dissected out, washed, 5µm thick section slides were prepared and stain with heamatoxyline-eosin and examined by light microscopy.

Pancreas: Normal rat's pancreas showed normal cellular structure. The histopathology of pancreas samples of rats treated with alloxan control group showed loss of normal architecture of cells. The histopathology of pancreas rats treated with standard drug metformin for 14 days showed normal architecture of the cells. The histopathology of pancreas samples of rats treated with ETT 200mg/kg for 14 days showed normal cells and ETT 400mg/kg showed normal cell structure.

3.4.6 Statistical analysis: Results were expressed as mean±SEM. The data were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests of different group. Statistical significance was considered at $P<0.05$ in all the cases.

4. RESULTS

4.1 Effect of Ethanolic extract of *Taxillus tomentosus* on blood sugar level (BSL) in alloxan induced diabetes in rats.

In an alloxan induced diabetic rats serum glucose level has significantly increased ($p<0.001$) after the 14 days in diabetic control rats when compared to normal groups. The values were shown in the table no.1.

Administration of ETT 200 and 400 mg/kg and Metformin 14.25 mg/kg orally for 14 days treatment were reduced significantly serum glucose level ($p<0.01$), ($p<0.001$) and ($p<0.001$) as compared to control groups.

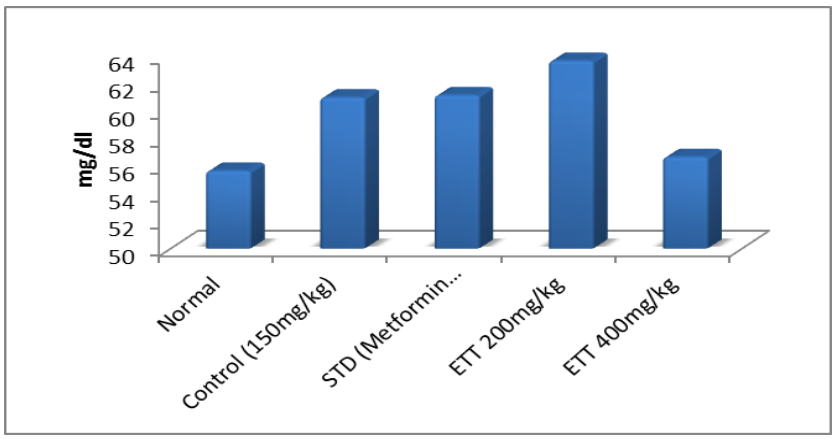

Fig.1; Effect of *Taxillus tomentosus* whole plant extract on glucose level at 0 day in alloxan induced diabetic rats.

All the values are Mean±SEM, n=6, One way ANOVA followed by multiple comparison of Dunnett's test, **p<0.01, ***p<0.001 as compared to control and [a]p<0.001as when compared to normal.

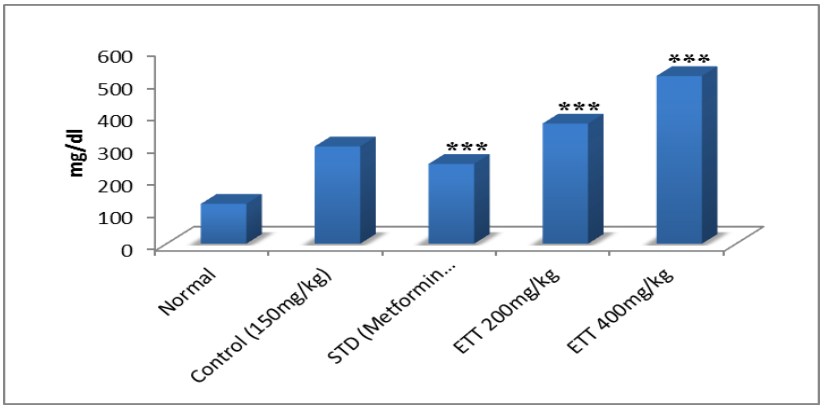

Fig.2; Effect of *Taxillus tomentosus* whole plant extract on glucose level at 1st day in alloxan induced diabetic rats.

All the values are Mean±SEM, n=6, One way ANOVA followed by multiple comparison of Dunnett's test, **p<0.01, ***p<0.001 as compared to control and [a]p<0.001as when compared to normal.

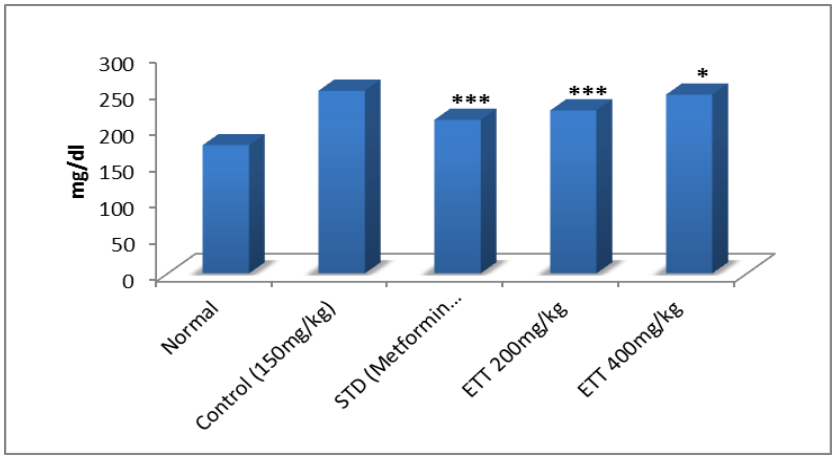

Fig.3; Effect of *Taxillus tomentosus* whole plant extract on glucose level at 7th day in alloxan induced diabetic rats.

All the values are Mean±SEM, n=6, One way ANOVA followed by multiple comparison of Dunnett's test, **p<0.01, ***p<0.001 as compared to control and [a]p<0.001as when compared to normal.

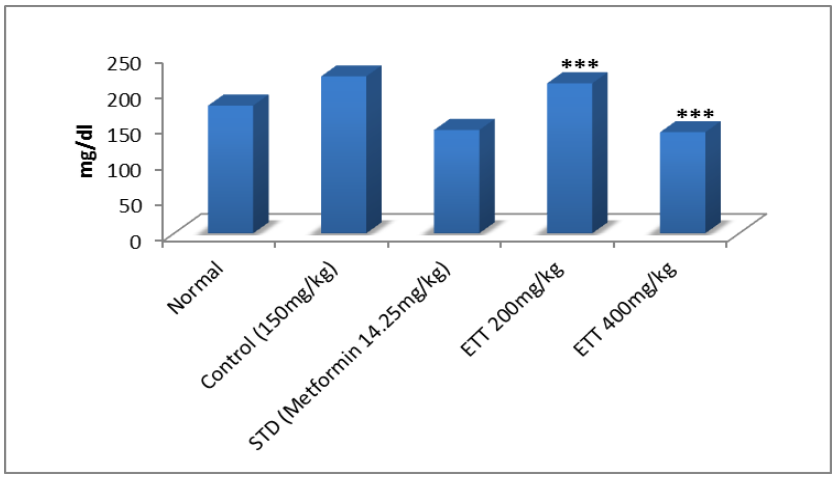

Fig.4; Effect of *Taxillus tomentosus* whole plant extract on glucose level at 14th day in alloxan induced diabetic rats.

All the values are Mean±SEM, n=6, One way ANOVA followed by multiple comparison of Dunnett's test, **p<0.01, ***p<0.001 as compared to control and [a]p<0.001as when compared to normal.

4.2 Effect of ETT on allodynia produced by tail immersion (cold water) in alloxan induced diabetes in rats.

No significant change in latency was observed in Normal group of animals throughout the study. For Control group of animals there was a gradual reduction in latency (sec) observed from 1st day to day 7th (2.842±0.449, p<0.001) till day 14th (8.978±1.080, p<0.01) indicating

the presence of allodynia. In the ETT group of animals, no significant lowering in pain latency was exhibited which implies the protective action of drug treatment on allodynia produced by cold water during the course of 14 day treatment. The values were shown in the table no.2.

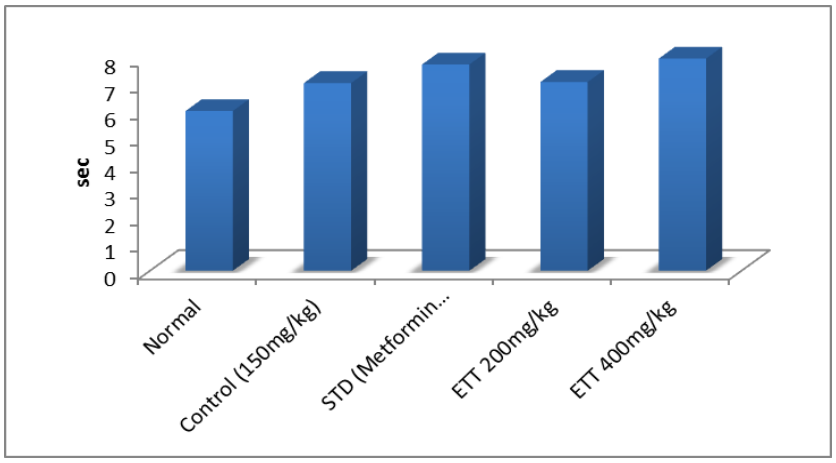

Fig.5; Effect of *Taxillus tomentosus* whole plant extract on neuroparameter at 1st day in alloxan induced diabetic rats (cold water test method).

All the values are Mean±SEM, n=6, One way ANOVA followed by multiple comparison of Dunnett's test, **p<0.01, ***p<0.001 as compared to control and [a]p<0.001as when compared to normal.

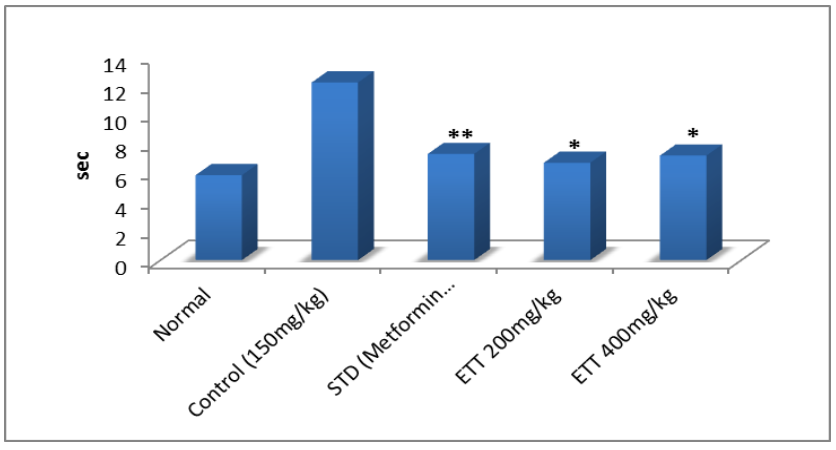

Fig.6; Effect of *Taxillus tomentosus* whole plant extract on neuroparameter at 7th day in alloxan induced diabetic rats (cold water test method).

All the values are Mean±SEM, n=6, One way ANOVA followed by multiple comparison of Dunnett's test, **p<0.01, ***p<0.001 as compared to control and [a]p<0.001as when compared to normal.

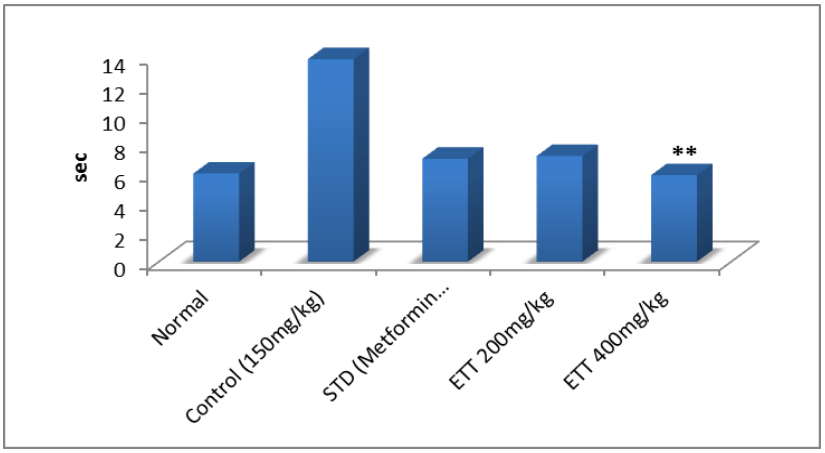

Fig.7; Effect of *Taxillus tomentosus* whole plant extract on neuroparameter at 14th day in alloxan induced diabetic rats (cold water test method).

All the values are Mean±SEM, n=6, One way ANOVA followed by multiple comparison of Dunnett's test, **p<0.01, ***p<0.001 as compared to control and [a]p<0.001as when compared to normal.

4.3 Effect of ETT on hyperalgesia produced by tail immersion (hot water) in alloxan induced diabetes in rats.

There was no change in tail flick latency (sec) observed in normal group of animals throughout the experiment. A gradual decline in the latency was observed in control group of animals from day 7th (6.333±0.49p<0.05) onwards which was observed minimum on day 14th (4.785±0.594, p<0.01), indicating the presence of neuropathic pain due to diabetes. ETT group of animal recorded a reduction in latency on day 14th (6.730±0.68 p<0.001) which was followed by increase in pain threshold time on subsequent days, indicating absence of algesia produced by tail immersion in hot water. The results were showed in table no.3.

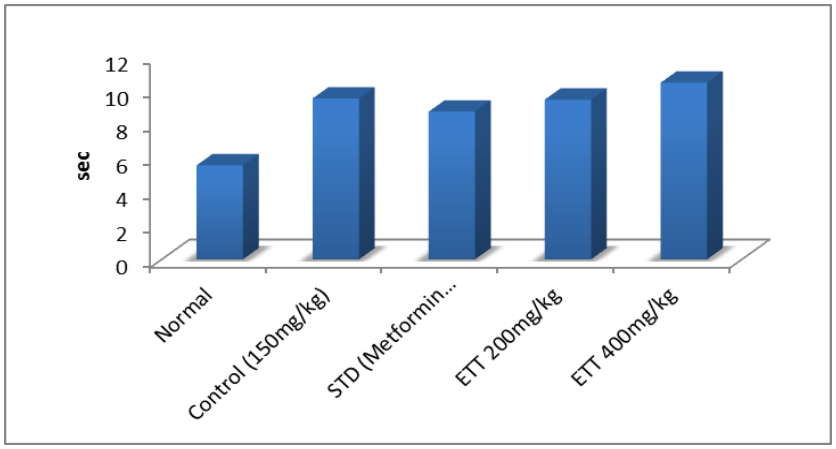

Fig.8; Effect of *Taxillus tomentosus* whole plant extract on neuroparameter at 1st day in alloxan induced diabetic rats (hot water test method).

All the values are Mean±SEM, n=6, One way ANOVA followed by multiple comparison of Dunnett's test, **p<0.01, ***p<0.001 as compared to control and [a]p<0.001as when compared to normal.

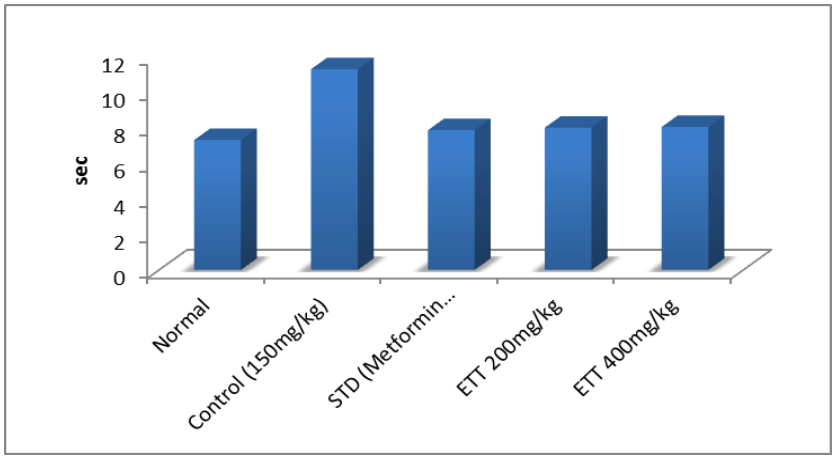

Fig.9; Effect of *Taxillus tomentosus* whole plant extract on neuroparameter at 7th day in alloxan induced diabetic rats (hot water test method)

All the values are Mean±SEM, n=6, One way ANOVA followed by multiple comparison of Dunnett's test, **p<0.01, ***p<0.001 as compared to control and [a]p<0.001as when compared to normal.

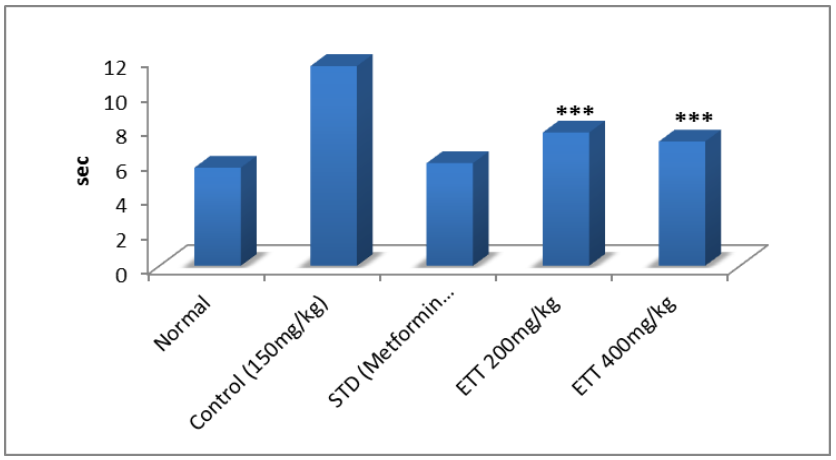

Fig.10; Effect of *Taxillus tomentosus* whole plant extract on neuroparameter at 14th day in alloxan induced diabetic rats (hot water test method).

All the values are Mean±SEM, n=6, One way ANOVA followed by multiple comparison of Dunnett's test, **p<0.01, ***p<0.001 as compared to control and [a]p<0.001as when compared to normal.

4.4 Effect of ETT on thermal hyperalgesia (hot plate) in alloxan induced diabetes in rats.

No significant change in latency was observed in Normal group of animals throughout the study. For Control group of animals there was a gradual reduction in latency (sec) observed from day 7th (2.33±0.21, p<0.05) till day 14th (3.067±0.29 p<0.001) where the pain was

observed to be maximum, indicating the presence of algesia by heat. In the drug treated group of animals, no significant lowering in pain latency was exhibited which implies the protective action of ETT on hyperalgesia produced in diabetic animals. The values were shown in table no.4.

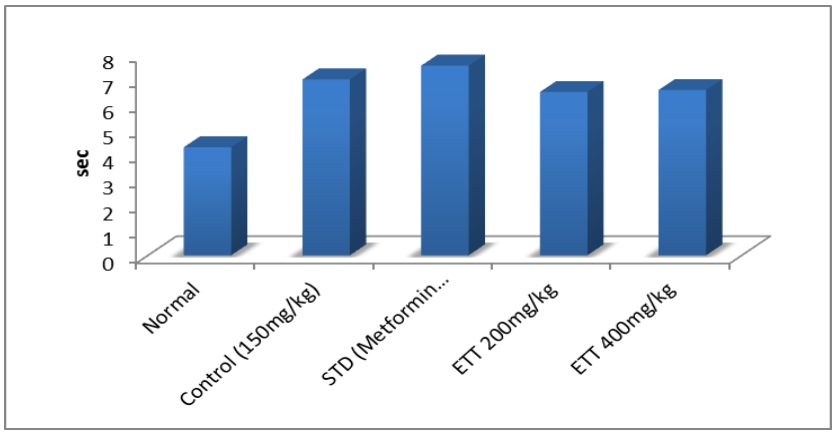

Fig.11; Effect of Taxillus tomentosus whole plant extract on neuroparameter at 1st day in alloxan induced diabetic rats (eddy's hot plate method).

All the values are Mean±SEM, n=6, One way ANOVA followed by multiple comparison of Dunnett's test, **p<0.01, ***p<0.001 as compared to control and [a]p<0.001as when compared to normal.

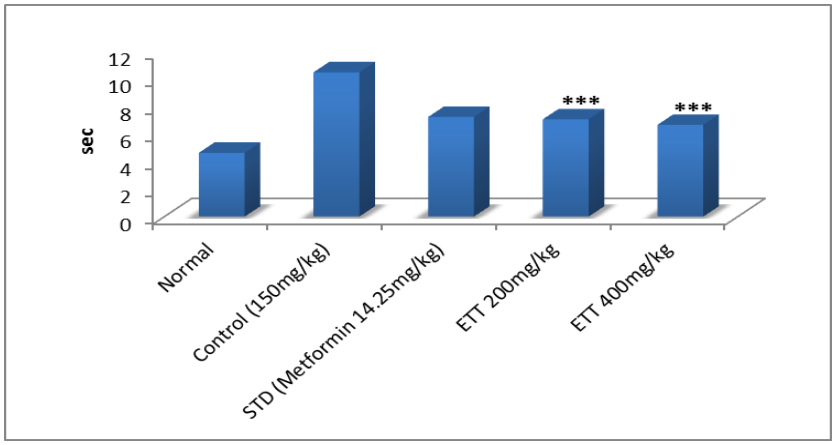

Fig.12; Effect of *Taxillus tomentosus* whole plant extract on neuroparameter at 7th day in alloxan induced diabetic rats (eddy's hot plate method).

All the values are Mean±SEM, n=6, One way ANOVA followed by multiple comparison of Dunnett's test, **p<0.01, ***p<0.001 as compared to control and [a]p<0.001as when compared to normal.

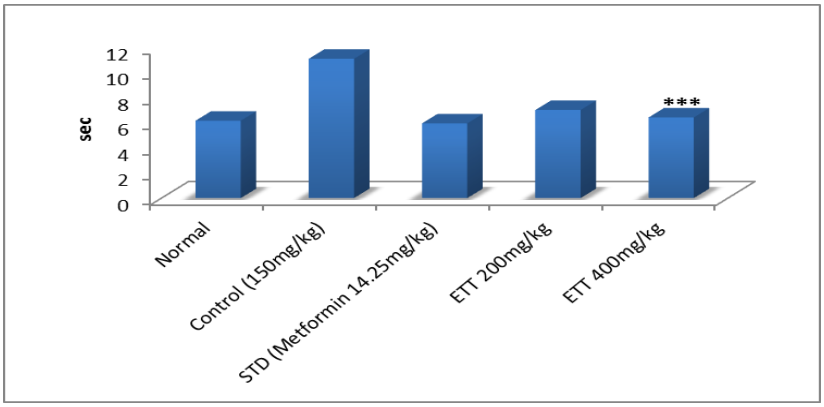

Fig.13; Effect of *Taxillus tomentosus* whole plant extract on neuroparameter at 14th day in alloxan induced diabetic rats (eddy's hot plate method).
All the values are Mean±SEM, n=6, One way ANOVA followed by multiple comparison of Dunnett's test, **p<0.01, ***p<0.001 as compared to control and [a]p<0.001as when compared to normal.

4.5 Effect of ETT on biochemical parameters produced in alloxan induced diabetes in rats: The biochemical parameters were estimated as per the standard procedure prescribed by the manufacturer's instruction manual provided in the standard kit using Auto analyzer. The results for the effect of the ETT in the mean values of serum biochemical parameters such as creatinine, uric acid, urea, SGOT, SGPT, ALP, cholesterol, HDL and triglycerides on normal and diabetic rats are presented in Table no 5, 6 & 7. Our data indicated that there were the mean values of above mentioned biochemical parameters were significantly higher in control rats as compared to the normal rats (P <0.001). Treatment of the diabetic rats with the ETT extract and metformin for 14 days (Groups III- V) caused a significant decrease in creatinine, uric acid, urea (p<0.001), (p<0.001), (p<0.001) and cholesterol, HDL, triglycerides (p<0.001) and SGOT, SGPT & ALP (p<0.01) as compared to control group.

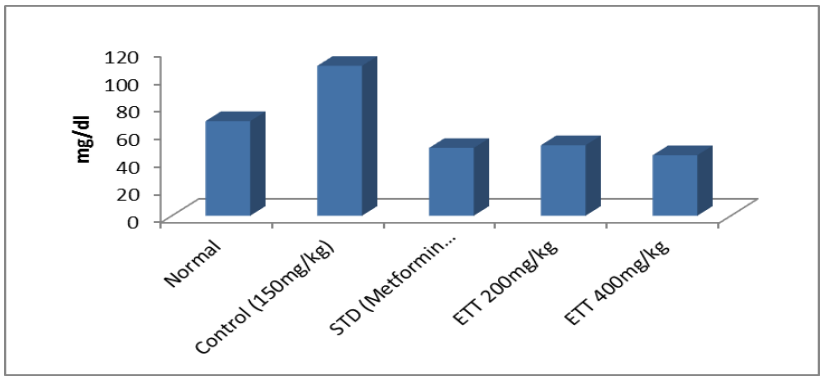

Fig.14; Effect of *Taxillus tomentosus* whole plant extract on triglycerides level at 14th day in alloxan induced diabetic rats.
All the values are Mean±SEM, n=6, One way ANOVA followed by multiple comparison of Dunnett's test, **p<0.01, ***p<0.001 as compared to control and [a]p<0.001as when compared to normal.

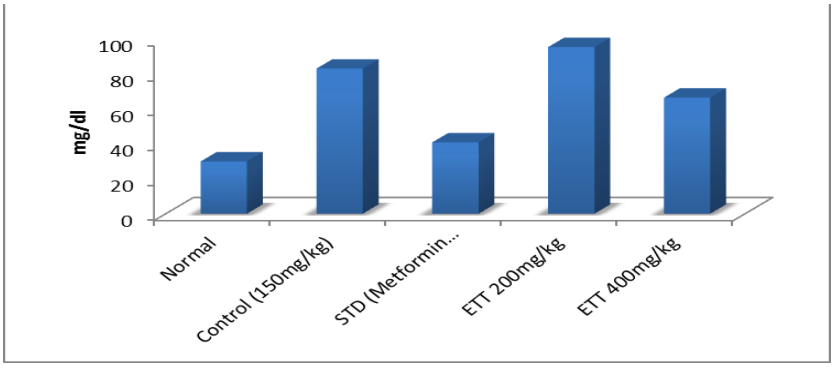

Fig.15; Effect of *Taxillus tomentosus* whole plant extract on cholesterol level at 14th day in alloxan induced diabetic rats.

All the values are Mean±SEM, n=6, One way ANOVA followed by multiple comparison of Dunnett's test, **p<0.01, ***p<0.001 as compared to control and [a]p<0.001as when compared to normal.

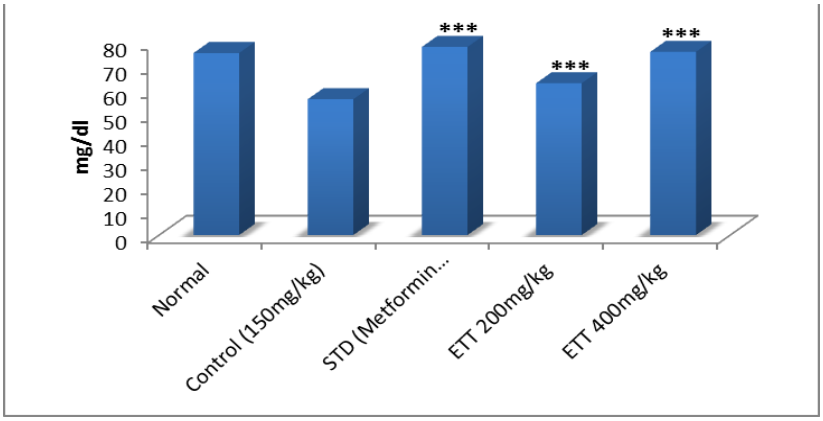

Fig.16; Effect of *Taxillus tomentosus* whole plant extract on HDL cholesterol level at 14th day in alloxan induced diabetic rats.

All the values are Mean±SEM, n=6, One way ANOVA followed by multiple comparison of Dunnett's test, **p<0.01, ***p<0.001 as compared to control and [a]p<0.001as when compared to normal.

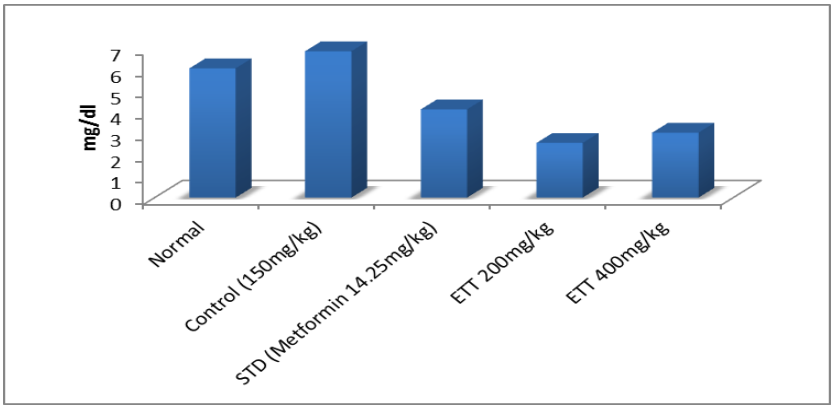

Fig.17; Effect of *Taxillus tomentosus* whole plant extract on Creatinine level at 14th day in alloxan induced diabetic rats.

All the values are Mean±SEM, n=6, One way ANOVA followed by multiple comparison of Dunnett's test, **p<0.01, ***p<0.001 as compared to control and [a]p<0.001as when compared to normal.

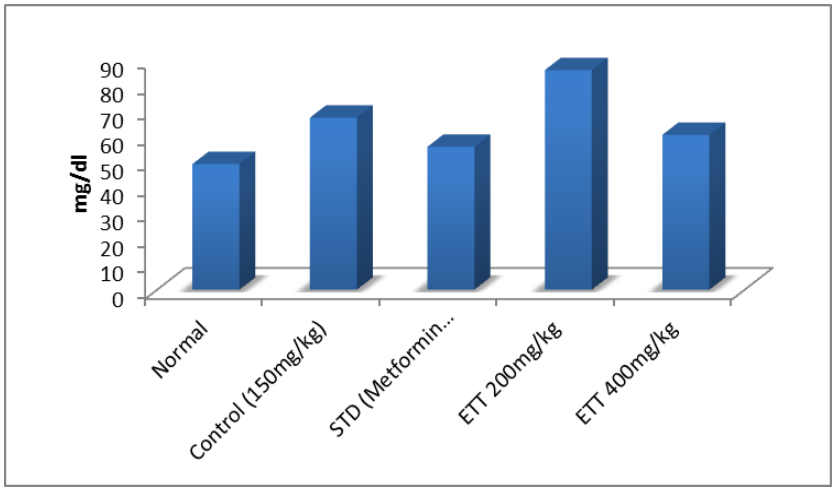

Fig.18; Effect of *Taxillus tomentosus* whole plant extract on Urea level at 14th day in alloxan induced diabetic rats.

All the values are Mean±SEM, n=6, One way ANOVA followed by multiple comparison of Dunnett's test, **p<0.01, ***p<0.001 as compared to control and [a]p<0.001as when compared to normal.

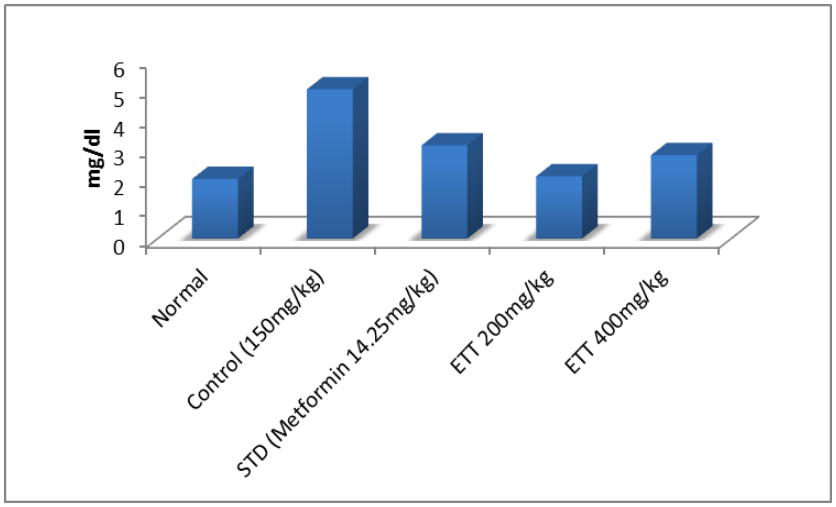

Fig.19; Effect of *Taxillus tomentosus* whole plant extract on Uric acid level at 14th day in alloxan induced diabetic rats.

All the values are Mean±SEM, n=6, One way ANOVA followed by multiple comparison of Dunnett's test, **p<0.01, ***p<0.001 as compared to control and [a]p<0.001as when compared to normal.

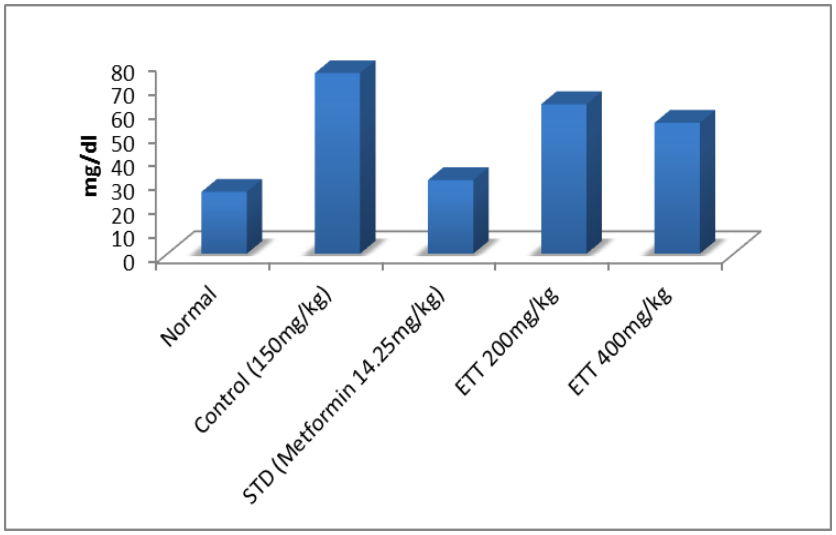

Fig.20; Effect of *Taxillus tomentosus* whole plant extract on ALP level at 14th day in alloxan induced diabetic rats.

All the values are Mean±SEM, n=6, One way ANOVA followed by multiple comparison of Dunnett's test, **p<0.01, ***p<0.001 as compared to control and [a]p<0.001as when compared to normal.

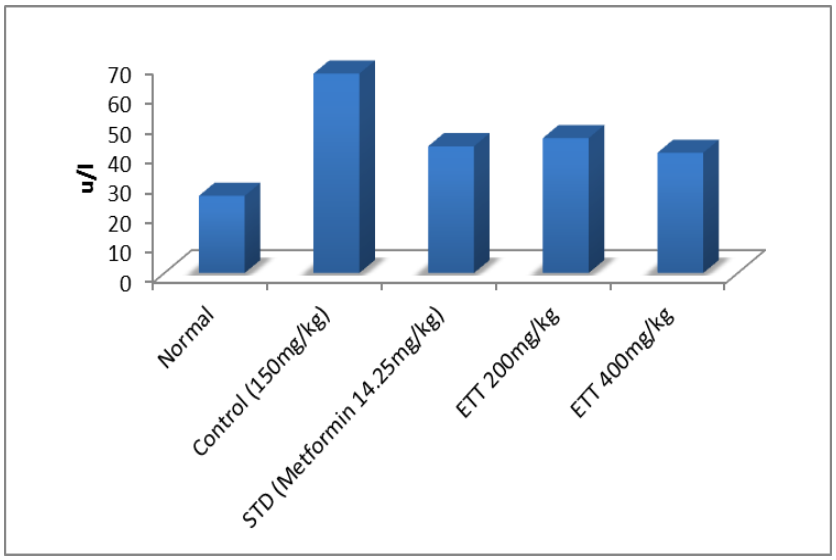

Fig.21; Effect of *Taxillus tomentosus* whole plant extract on SGOT level at 14th day in alloxan induced diabetic rats.

All the values are Mean±SEM, n=6, One way ANOVA followed by multiple comparison of Dunnett's test, **p<0.01, ***p<0.001 as compared to control and [a]p<0.001as when compared to normal.

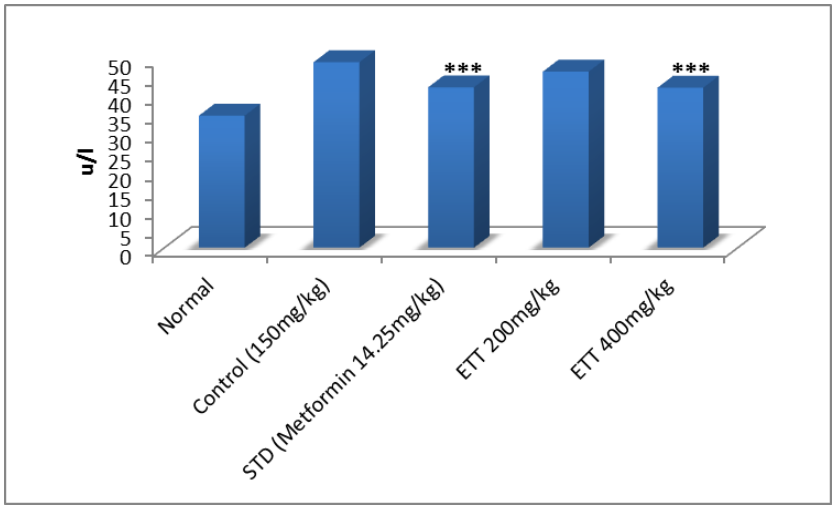

Fig.22; Effect of *Taxillus tomentosus* whole plant extract on SGPT level at 14th day in alloxan induced diabetic rats.

All the values are Mean±SEM, n=6, One way ANOVA followed by multiple comparison of Dunnett's test, **p<0.01, ***p<0.001 as compared to control and [a]p<0.001as when compared to norml.

4.5 Histopathology: At the end of the study period (on 14th day), animals from all the five groups were anesthetized under mild ether anesthesia and sacrificed; pancreas was dissected out, washed, 5µm thick section slides were prepared and stain with heamatoxyline-eosin and examined by light microscopy.

Pancreas: Normal rat's pancreas showed normal cellular structure. The histopathology of pancreas samples of rats treated with alloxan control group showed loss of normal architecture of cells. The histopathology of pancreas rats treated with standard drug metformin for 14 days showed normal architecture of the cells. The histopathology of pancreas samples of rats treated with **ETT 200mg/kg** for 14 days showed normal cells and **ETT 400mg/kg** showed normal cell structure. The results were showed in figure 23.

A. Normal group

B. Control group

C. Metformin (standard) treated group

D. ETT 200mg/kg group

E. ETT 400mg/kg group

Table.1: Effect of *Taxillus tomentosus* whole plant extract on blood glucose level in alloxan induced diabetic rats

Groups	Blood glucose level in mg/dl			
	0 day	1st day	7th day	14th day
Normal	55.66±1.54	122.86±1.59	176.35±1.25	179.46±1.31
Control	61±1.86	300.25±1.38a	251.21±1.09a	220.53±1.22a
Standard	61.16±1.16	246.28±0.99***	211.16±1.25***	145.15±1.17***
ETT 200mg/kg	63.66±2.47	370.7±1.17***	224.01±1.4***	210.74±1.32***
ETT 400mg/kg	56.66±1.40	517.31±1.52***	245.79±1.25*	142.08±1.31***

Values are mean±SEM; n=6 *p<0.01 **p<0.001 ***p<0.0001 compared with diabetic control.

Table.2: Effect of *Taxillus tomentosus* whole plant extract on Neuroparameters in alloxan induced diabetic rats

Groups	Cold water test method		
	1st day	7th day	14th day
Normal	6.01±0.47	5.83±0.72	6.02±1.02
Control	7.06±0.78c	12.2±0.77b	13.83±1.1a
Standard	7.76±0.78	7.29±0.88**	7.03±0.79***
ETT 200mg/kg	7.1±0.6	6.69±0.91*	7.22±0.88**
ETT 400mg/kg	7.98±0.8	7.18±0.77*	5.91±0.92**

Values are mean±SEM; n=6 *p<0.01 **p<0.001 ***p<0.0001 compared with diabetic control.

Table.3: Effect of *Taxillus tomentosus* whole plant extract on Neuroparameters in alloxan induced diabetic rats

Groups	Hot water test method		
	1st day	7th day	14th day
Normal	5.55±0.96	7.3±0.37	5.68±0.69
Control	9.5±1.06c	11.28±0.7a	11.56±0.86a
Standard	8.72±0.56	7.85±0.83**	5.94±0.89***
ETT 200mg/kg	9.43±1.17	7.99±0.8***	7.71±0.69***
ETT 400mg/kg	10.43±1.02	8.03±0.53**	7.19±0.84***

Values are mean±SEM; n=6 *p<0.01 **p<0.001 ***p<0.0001 compared with diabetic control

Table.4: Effect of *Taxillus tomentosus* whole plant extract on Neuroparameters in alloxan induced diabetic rats

Groups	Eddy's hot plate method		
	1st day	7th day	14th day
Normal	4.30±0.34	4.63±0.39	6.15±0.4
Control	**7**±0.75c	10.47±0.63a	11.07±0.42a
Standard	7.55±0.44	7.23±0.66*	5.94±0.76***
ETT 200mg/kg	6.5±0.7	7.06±0.54***	7±0.93**
ETT 400mg/kg	6.58±0.52	6.66±0.46***	6.41±1.06***

Values are mean±SEM; n=6 *p<0.01 **p<0.001 ***p<0.0001 compared with diabetic control.

Table.5: Effect of *Taxillus tomentosus* whole plant extract on Biochemical parameters in alloxan induced diabetic rats

Groups	Biochemical parameters		
	Triglycerides	Cholesterol	HDL
Normal	68.39±1	29.96±1.25	75.51±1.04
Control	108.37±1a	83.2±1a	56.34±1.15a
Standard	49±1.07***	40.92±1.37***	78.05±1.03***
ETT 200mg/kg	50.94±1.09***	95.53±1***	62.99±1.14***
ETT 400mg/kg	43.76±1***	66.56±0.92***	75.99±1***

Values are mean±SEM; n=6 *p<0.01 **p<0.001 ***p<0.0001 compared with diabetic control

Table.6: Effect of *Taxillus tomentosus* whole plant extract on Biochemical parameters in alloxan induced diabetic rats

Groups	Biochemical parameters		
	Creatinine	Urea	Uric acid
Normal	6.05±0.76	49.21±1.39	2±0.58
Control	6.85±0.82a	67.36±1.04a	5.01±0.5a
Standard	4.13±0.75*	55.96±1.23**	3.13±0.33*
ETT 200mg/kg	2.56±0.48***	86.02±1.09*	2.09±0.35**
ETT 400mg/kg	3.04±0.66***	60.66±1.08**	2.8±0.61*

Values are mean±SEM; n=6 *p<0.01 **p<0.001 ***p<0.0001 compared with diabetic control

Table.7: Effect of *Taxillus tomentosus* whole plant extract on Biochemical parameters in alloxan induced diabetic rats

Groups	Biochemical parameters		
	SGOT	SGPT	ALP
Normal	25.9±1.31	34.82±.96	25.86±0.81
Control	66.96±0.7a	48.91±1.05a	75.51±0.89a
Standard	42.56±1.13***	42.3±1.17***	30.78±0.99***
ETT 200mg/kg	45.3±1.09***	46.44±0.85	62.55±0.89***
ETT 400mg/kg	40.4±1.01***	42.2±0.86***	54.70±0.76***

Values are mean±SEM; n=6 *p<0.01 **p<0.001 ***p<0.0001 compared with diabetic control

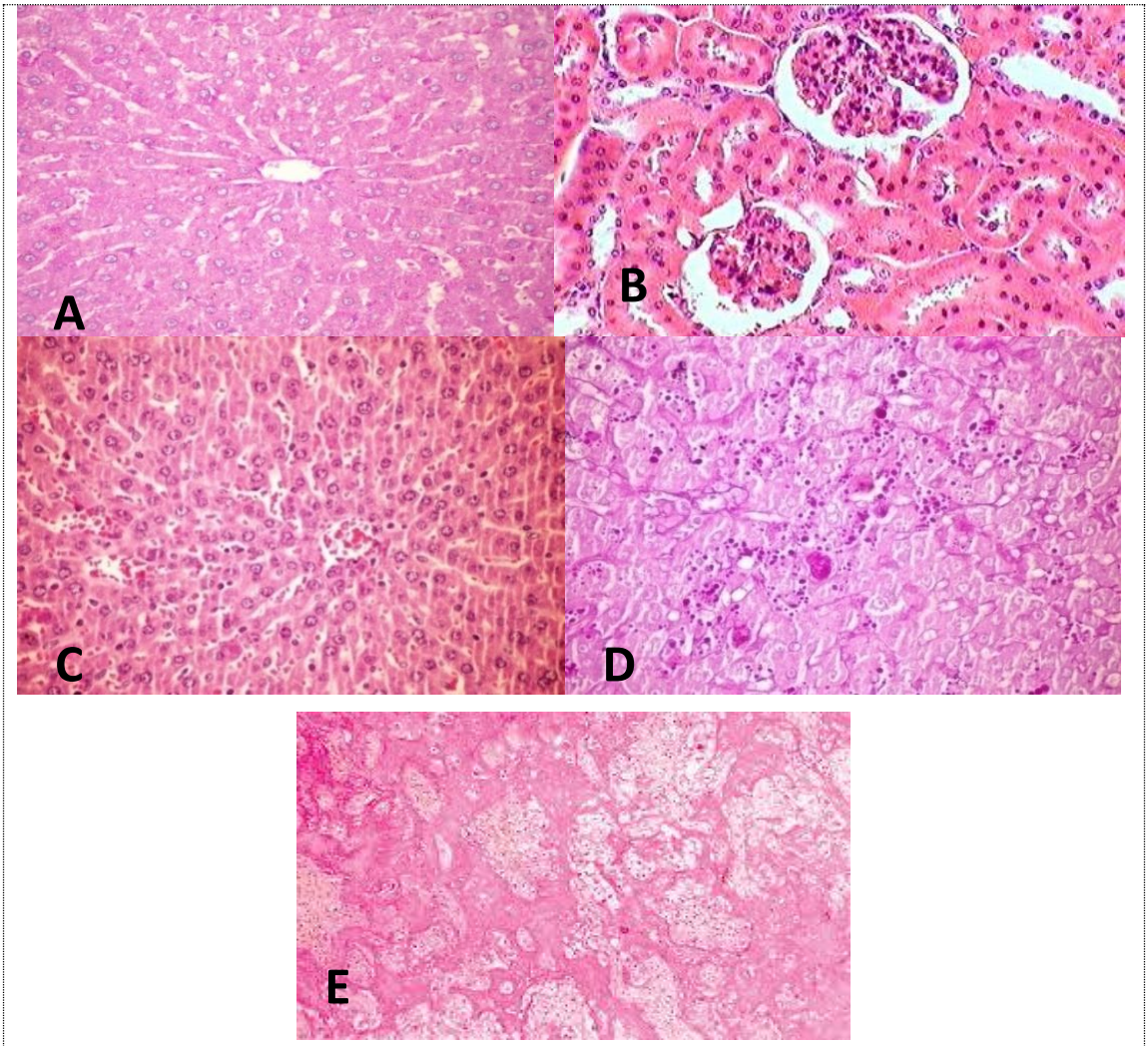

Fig.23: Histophathology of pancreas.

A. Normal group

B. Control group

C. Metformin (standard) treated group

D. ETT 200mg/kg group

E. ETT 400mg/kg group

5. DISCUSSION

The acute toxicity test of ETT in mice produced no death or signs of toxicity even at the dose of 2000 mg/kg which shows that the extract was well tolerated and the test doses safe in the animals.

The antidiabetic activity of ETT was evaluated in alloxan induced diabetic rats by testing its effect on fasting blood glucose level using auto analyzer (AccuCheckActive®) & glucose kit. The fasting blood sugar test is a carbohydrate metabolic test which measures plasma or

blood glucose levels after a fast (usually 8–12 h). During fasting the body stimulates the release of the hormone glucagon, which in turn releases glucose into the blood through catabolic processes. Normally, the body produces and processes insulin to counteract the rise in glucose levels but in diabetes, this process does not occur and tested glucose levels normally remain high. Alloxan is one of the usual substances used for induction of diabetes mellitus apart from streptozotocin and has a destructive effect on the beta (β) cells of the pancreas as previously reported. Pancreas is the primary organ involved in sensing the organism's dietary and energetic states via glucose concentration in the blood and in response to elevated blood glucose, insulin is secreted .However, alloxan causes diabetes through its ability to destroy the insulin-producing-cells of the pancreas. When there are not enough available beta-cells to supply sufficient insulin to meet the needs of the body, insulin-dependent diabetes results.

A strong relationship involves between glycaemia and diabetic microvascular complications in both type 1 and type 2 diabetes production of superoxide due to oxidative stress in diabetes may be cause for vascular and neuronal complications of painful neuropathy. In diabetes, intracellular hyperglycemia causes abnormalities in blood flow and increased vascular permeability. Quantitative and qualitative abnormalities of extracellular matrix contribute to an irreversible increase in vascular permeability. With microvascular cell loss occurs in part as a result of programmed cell death. Hyperglycemia may also decrease production of trophic factors for endothelial and neuronal cells. Together, these changes lead to edema, ischemia and hypoxia induced neovascularization in the retina, proteinuria, messengial matrix expansion, and glomerulosclerosis in the kidney and multifocal axonal degeneration in peripheral nerves. Impaired blood flow also seems to contribute to noxious stimulus hypersensitivity. Oxidative stress related reduction in perfusion is thought to play a part in cardiac autonomic dysfunction and also in small fiber sensory neuropathy. Alloxan and the products of its reduction, dialuric acid, establish a redox cycle with 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 oxidant species (ROS) with a simultaneous massive increase in cytosolic calcium concentration cause rapid Beta cell destruction. Early pharmaceutical intervention against the long-term consequences of hyperglycemia induced cross-linking prevents the development of severe late complications of diabetes.

Hyperglycemia has also been recently implicated in initiation and development of various types of diabetic complications. Nephropathy is one of these serious microvascular complications that have been observed in diabetic individuals. In addition, blood urea, uric acid and creatinine concentrations were increased among uncontrolled diabetic individuals and this increase could be a result of impaired renal function due to an increased blood glucose level. Our results revealed for the first time that the mean values of these end products in the serum increased in untreated diabetic rats, while they significantly decreased after the administration of ETT. Thus, this extract might improve renal function which, in turn, leads to reduction in these end products. It was reported that diabetic individuals had lower serum creatinine concentrations as well as higher serum uric acid and urea levels than nondiabetic individuals. Thus, the reduction in urea and creatinine levels probably can be explained by a reduction in blood glucose level. The SGOT, SGPT and ALP were significantly decreased with administration of ETT thus indicating the plant has hepatoprotective property.

Moreover, high levels of serum uric acid, urea and creatinine may act as a marker of kidney problems. Thus, it is possible to suggest that this extract might play an important role in reducing risk of kidney problems as well as neuroprotective by lowering both hyperalgesia and allodynia as well as serum urea, uric acid, creatinine The hyperalgesic response in tail-withdrawal test is generally attributed to central mechanisms whereas the hyperalgesic response on hot plate is attributed to the combination of both central and peripheral mechanisms. The beneficial effects that have been seen for the first time in our study are indications of safety of ETT extract. Further research needs to carry out in order to explore the actual component responsible for protective effects against diabetic complications and for antidiabetic activity.

6. CONCLUSION

In conclusion, the presented data indicate that administration of the ETT plant to rats with metformin induced diabetic mellitus and prevented the diabetic complication, supporting folk information regarding antidiabetic activity of the plant. The mechanism underlying this effect is still unknown, but is apparently related to insulin production or sensitization of tissues to insulin and lowering of glucose concentration in blood. The protective effect against diabetic complications may be contributing to the recovery of tissues damage. These effects could conclude the antidiabetic property of Taxillus tomentosus.

7. SUMMARY

The present work was carried out evaluate antidiabetic activity of *Taxillus tomentosus* in this study the dried powder of whole plant of *Taxillus tomentosus* was subjected to the extraction process by Soxhlet apparatus using 95% of ethanol for 72 hours. The extract was obtained and the percentage yield was 11.98%. Some of extract was used for the preliminary phytochemical screening and rest of the part was utilized for pharmacological screening.

The Phytochemical screening of plant extract shows the presence of carbohydrate, protein, flavonoids, alkaloids, steroids and saponins.

Evaluation of antidiabetic activity was assessed by using alloxan induc**e** diabetic model. Acute diabetes was induced by administration of alloxan (150mg/kg i.p, at once) in Wister albino rats.

The oral administration of ethanolic extract of Taxillus tomentosus significantly decreases the evaluated serum glucose in diabetic rats Administration of ETT 200 and 400 mg/kg and Metformin 14.25 mg/kg orally for 14 days treatment were reduced significantly serum glucose level as compared to control groups.

Blood urea, uric acid and creatinine concentrations were increased among diabetic rats and this increase could be a result of impaired renal function due to an increased blood glucose level. They were significantly decreased after the administration of ETT. Thus, this extract might improve renal function.

The cholesterol, Triglycerides and HDL were significantly decrease with oral administration of ETT 200mg/kg and 400mg/kg.

Both hyperalgesia and allodynia was lowered with oral administration of ETT extract. The SGOT, SGPT and ALP were significantly decreased with administration of ETT thus indicating the plant has hepatoprotective property.

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