

Anti-hyperglycemic Potential of *Trichuriella monsoniae*

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Abstract

The anti-hyperglycemic activity of methanolic extract fraction of *Trichuriella monsoniae* (TM) was evaluated in Streptozotocin induced diabetic rats. Methanolic extract was fractionated and subjected to antidiabetic studies in streptozotocin induced diabetic rats to identify the most potent bioactive fraction. Oral administration of fractions of METM at 20 & 40 mg/kg b.w. significantly reduced the fasting blood glucose levels in diabetic rats. Among the fractions, n- butyl alcohol fraction (BLTM) at 40 mg/kg b.w. was found to be more effective. Further, in sub-acute study, BLTM significantly reduced the elevated level of glucose, cholesterol, triglycerides, insulin, SGPT, ALP, creatinine in serum and increased the diminished body weight and total protein and insulin level in serum in streptozotocin induced diabetic rats. Increased glycogen content was observed in liver and skeletal muscle of the diabetic rats after 28 days of the treatment. BLTM ameliorated the histological damage of islets of langerhans. BLTM also elicited a significant antioxidant effect, which was evident from its ability to inhibit lipid peroxidation and also by scavenging the DPPH radical. The results of the study clearly indicate that BLTM exerts potent antidiabetic activity.

Keywords: Anti-hyperglycemic activity, *Trichuriella monsoniae*, Streptozotocin, MDA, DPPH, HPTLC

Introduction

Diabetes is a chronic metabolic disorder, characterized by absolute or relative deficiency in insulin secretion and/or insulin action associated with chronic hyperglycemia and disturbances of carbohydrate, lipid and protein metabolism. The metabolic changes occur in diabetes lead to develop various complications including macro and micro vascular dysfunctions [1]. The prevalence of diabetes mellitus is rapidly increasing world wide. By the year 2030, diabetes mellitus is expected to affect almost 30% of world's population – an estimated 366 million people [2]. Oxidative stress has been shown to have a significant effect in the causation of diabetes as well as diabetes related complications in human beings [3]. Therefore, the anti diabetic drugs with antioxidant potential are beneficial in the management of diabetes.

Trichuriella monsoniae (L.f.) Bennet belongs to the family Amaranthaceae is an annual prostrate herb, rooting at nodes is commonly found in gravelly and sandy localities in scrub jungles. Leaves are linear (or) filiform, clustered at nodes, subsessile, acute. Flowers appear as cylindrical spikes with pink colour. Traditionally, the whole plant is used to treat diabetes, urinary calculi, wounds, sore throat and as hepatoprotective and diuretic. The plant is commonly known as Erra pindipulu, pedda telaga chettu [4].

Since the whole plant of *Trichuriella monsoniae* is claimed to be useful in the treatment of diabetes and the preliminary investigation with its methanolic extract exhibited a significant antihyperglycemic activity in streptozotocin induced diabetic rats [5], the present study

was designed to explore the antihyperglycemic potential of methanolic extract fractions.

Materials and Methods

Collection of Plant Material

Whole plant of *Trichuriella monsoniae* was collected in and around Kakatiya University Campus, Warangal, Andhra Pradesh in the month of November, 2010. The plant material was authenticated by Dr. V.S. Raju (taxonomist), Dept. of Botany, Kakatiya University, Warangal. The voucher specimen (KU/UCPSC/27/2010) of this plant material has been retained in the Department of Pharmacognosy and Phytochemistry for future reference.

Preparation of Methanolic Extract and its Fractions

The whole plant was collected, washed in running water, dried under shade and then ground into coarse powder for the maceration process with methanol at room temperature. After exhaustive extraction, the methanolic extract was concentrated under reduced pressure to yield brownish green coloured mass. The methanolic extract (METM) was suspended in water and fractionated with Toluene, Ethyl acetate and 1-butanol in succession.

Animals

Wister albino rats (150-180g) of either sex were procured from Mahaveer enterprises, Hyderabad. They were maintained in a

controlled environment and temperature (24 ± 5 C with 12-h of light/dark cycle). Rats were fed with standard laboratory diet and water was given *ad libitum*. All experimental protocols were approved by the Institutional Animal Ethical Committee (CPCSEA Reg.No. 146/2009).

Chemicals

Streptozotocin (STZ) and 1, 1, 3, 3- tetra ethoxy propane (TEP) were purchased from Sigma- Aldrich (Germany), where as Thio barbituric acid (TBA) and 1, 1- Di-phenyl-2-Picryl hydrazyl (DPPH) from Hi-media (Mumbai, India), and Trichloro acetic acid (TCA) from MERCK (Mumbai, India). Glibenclamide is a generous gift from Orchid laboratories, Chennai, India. Glucose, serum glutamate pyruvic transaminase (SGPT), alkaline phosphatase (ALP), total protein, creatinine, triglycerides, total cholesterol levels were studied by Merck Micro Lab 300 analyzer by using Merck analytical Kits. All other chemicals used were of analytical grade.

Acute toxicity studies (OECD 2001)

The acute toxicity studies were conducted according to OECD 420 guidelines (OECD 2001). The rats were allowed to food and water *ad libitum* and were observed continuously for 24 hrs for their behavioral, neurological changes. After period of 48 & 72 h, animals were observed for signs of lethality (or) for death.

Preliminary Phytochemical screening

Preliminary phytochemical screening was done for n-butanol fraction of *T.monsoniae* by test tube reactions and TLC methods.

Evaluation of antidiabetic activity of the different fractions of METM in STZ induced diabetic rats

Acute treatment

NIDDM (Non Insulin Dependent Diabetes Mellitus) was induced by intraperitoneal injection of streptozotocin at a dose of 90 mg/kg to 48±2h old neonatal rats. After 8 wks of STZ administration, the diabetic rats (glucose level > 180mg/dl) were separated and divided into 8 groups of six animals in each and treated orally in the following manner (Angel *et al.*, 1996) Group I : diabetic control rats administered with 5% gum acacia, Group II: Diabetic rats administered with standard drug glibenclamide (10 mg/kg), Group III& IV, V& VI and VII & VIII were treated with two test doses, 20 and 40 mg/kg b.w. of each of toluene, ethyl acetate, 1-butanol fractions respectively. Blood samples were collected just before and 2, 4, 6, 8, 12 and 24 h after administration of the test samples and were analysed for blood glucose content by using glucose oxidase method [6].

Sub acute treatment

The diabetic rats (glucose level >180mg/dl) were divided into 3 groups of 6 animals in each. Group I: Diabetic control rats administered gum acacia (5%) p.o; Group II: Diabetic rats administered glibenclamide at a dose of (10mg/kg) p.o; Group III: Diabetic rats administered BLTM at a dose of 40 mg/kg p.o. The animals of all the groups were treated with the respective study material once in a day for 28 days. During the study period, the body weight of the animals and the fasting blood glucose levels were recorded on the day 1, 7, 14, 21 and 28. Blood samples were collected by puncturing retro orbital plexus for estimation of various biochemical parameters like cholesterol, triglycerides, insulin, SGPT, ALP, creatinine, total protein. At the end of the study, the liver and skeletal muscle samples were collected from the animals for estimation of glycogen content by colorimetric method [7]. Pancreas was isolated to study the histopathological changes.

Determination of Antioxidant activity

Antioxidant activity of BLTM was measured by following two methods:

Free radical scavenging assay

1 ml of 0.1 mM solution of DPPH in methanol was added to 2.5ml of the test extract in methanol (10 -100 µg/ml). The reaction mixture was then allowed to stand at room temperature in a dark chamber for 30 min. After 30 min, absorbance was measured at 517nm using UV-Visible spectrophotometer. Ascorbic acid was used as a standard. The scavenging activity of DPPH radical (%) was calculated from the following equation:

$$\% \text{ Scavenging activity} = \frac{[A_{\text{Control}} - A_{\text{Extract}}] / A_{\text{Control}} \times 100.}$$

Lower absorbance of the reaction mixture indicates higher free radical scavenging activity [8].

Lipid peroxidation assay

The amount of lipid peroxidation product (MDA) present in the serum samples drawn from streptozotocin induced diabetic control, BLTM and glibenclamide treated diabetic rats after 28 days of the study was estimated by the thiobarbituric acid reactive substances (TBARS) method [8]. Serum samples (0.5ml) were transferred into clean, dry and labeled 10 ml test tubes. To these test tubes 0.5 ml of 30% trichloroacetic acid was added to precipitate the proteins and 100 µl of 1% thiobarbituric acid was added and all the test tubes were covered with aluminium foil and heated on a water bath at 95° C for 1h. The test tubes were cooled in an ice bath for about 10 min and centrifuged at 3000 rpm for about 10-15 min. Then 100 µl of supernatants were collected and transferred in to clean dry test tubes and diluted to 4 ml with distilled water, mixed well and read the absorbance of solutions at 540 nm using spectrophotometer (UV-Spectrophotometer; Elico-SL 159, Germany). The MDA values were calculated using 1, 1, 3, 3- tetra

ethoxy propane as the standard and expressed as nmoles of MDA/ml of serum.

High performance Thin Layer Chromatography

(HPTLC) fingerprinting profile of BLTM

A densitometry HPTLC analysis was performed for the development of characteristic fingerprinting profile of the bio active extract, BLTM. The extract was dissolved in methanol 1mg/ml and 25µl of the sample was loaded on precoated TLC plate of silica gel of 60 F254 (E.Merck, India) in the form of a band using Linomat IV Automatic Spotter (CAMAG, Switzerland). TLC plate was developed using chloroform: methanol (9:1) as a solvent system. The developed plate was air dried and observed under UV light. Then the plates were scanned in CAMAG TLC scanner and the peaks were recorded at a wavelength of 254nm. The Rf values and percentage of separated compounds were determined.

Statistical analysis

All the values were expressed as Mean \pm SD. The data was statistically evaluated using one way analysis of variance (ANOVA) followed by Dunnetts t-multiple comparison test using Graph pad Prism 3 computer software. P value of 0.05 or less was considered to be significant.

Results

Preliminary phytochemical screening

Preliminary phytochemical investigation reveals that the butanol fraction of *Trichuriella monsoniae* contains flavonoidal and steroidal/ terpenoidal glycosides, saponins and phenolic compounds.

Acute toxicity studies

Acute toxicity studies reveal that there were no toxic effects and no lethality in the animals treated with TLTM, EATM and BLTM. They were found safe up to dose of 2000mg/kg body weight. Hence, two test doses i.e. 20 & 40 mg/kg b.w of the fractions, which are less than that of the effective methanolic extract dose (50mg/kg b.w) in antihyperglycemic studies conducted on STZ induced diabetic rats, were selected for the study.

Antihyperglycemic effect of different fractions of METM

TLTM, EATM and BLTM at both the test doses i.e. 20 & 40mg/kg b.w reduced the blood glucose levels after 2h of administration and continued the effect up to 12h. Among the three fractions, BLTM significantly suppressed the elevated blood glucose levels after 6h of administration at both the test doses. The prominent antihyperglycemic effect was observed for BLTM at 40mg/kg b.w and was comparable with reference drug glibenclamide at 10mg/kg b.w. While the other two fractions i.e. TLTM and EATM at both the test dose marginally reduced the fasting blood glucose levels after 6h of administration. The results were represented in Table 1

Table 1: Effect of *T.monsoniae* fractions on blood glucose level in streptozotocin induced diabetic rats

Blood glucose level (mg/dl) at different hours after the treatment								
Group	Dose	0 hr	2 hr	4hr	6hr	8 hr	12 hr	24 hr
Diabetic control	---	218.9 \pm 2.7	222.5 \pm 7.1	225.6 \pm 4.0	227.6 \pm 2.2	232.1 \pm 5.4	235.8 \pm 1.4	237.8 \pm 2.9
Glibenclamide	10mg	237.3 \pm 5.2	189.7 \pm 6.4*	167.2 \pm 3.9**	128.1 \pm 3.4**	154.3 \pm 3.7**	170.1 \pm 6.2**	184.0 \pm 5.0
TLTM	20mg	238.3 \pm 2.8	229.6 \pm 4.2**	212.1 \pm 5.4**	185.7 \pm 5.6**	190.8 \pm 6.7**	199.1 \pm 7.6**	207.3 \pm 3.3
TLTM	40mg	247.1 \pm 4.0	221.2 \pm 4.5*	199.3 \pm 6.8**	180.0 \pm 3.0**	188.8 \pm 2.6**	197.0 \pm 3.1**	203.0 \pm 5.2
EATM	20mg	227.3 \pm 8.1	214.6 \pm 8.3**	201.1 \pm 4.2**	189.5 \pm 6.3**	191.7 \pm 4..7**	196.1 \pm 4.4**	205.5 \pm 4.3
EATM	40mg	186.7 \pm 5.1	178.2 \pm 3.5**	174.8 \pm 6.3**	165.1 \pm 4.7**	169.0 \pm 3.7**	177.5 \pm 3.6**	180.6 \pm 6.4
BLTM	20mg	255.0 \pm 5.5	218.4 \pm 6.6*	194.7 \pm 4.8**	150.6 \pm 3.3**	167.5 \pm 6.3**	182.0 \pm 4.2**	216.1 \pm 5.8
BLTM	40mg	244.3 \pm 8.3	194.6 \pm 7.5	156.9 \pm 2.9**	124.9 \pm 3.5**	142.2 \pm 3.0**	162.6 \pm 5.2**	200.5 \pm 6.9

Effect of BLTM on different parameters in sub acute study (28 days)

Body weight

Diabetic control rats showed continuous reduction in their body weight during 28 days. BLTM (40 mg/kg b.w) treated diabetic rats significantly ($P < 0.01$) improved their body weight after 7 days of treatment and the effect was observed till the end of the study (28 days). This effect was well comparable with that of reference drug glibenclamide at 10mg/kg b.w. The results are depicted in Table 2.

Table2: Effect of BLTM on body weight and blood glucose level in streptozotocin induced diabetic rats

Group	Body weight (g)					Blood glucose (mg/dl)				
	Days of treatment					Days of treatment				
	1	7	14	21	28	1	7	14	21	28
Diabetic control	171.5±3.0	170.1±3.2	168.5±2.6	167.5±2.7	164.8±2.7	240.6±4.6	245.0±4.4	248.3±2.8	250.3±2.7	253.8±3.7
Glibenclamide (10mg/kg)	157.8±5.7	161.8±4.0	163.1±4.7	169.0±5.2	171.1±6.4	245.1±7.5	200.1±6.2**	168.0±9.2**	147.1±4.0**	122.8±6.1**
BLTM (40mg/kg)	166.6±6.0	169.6±5.8	172.0±6.5	176.5±6.4	182.1±7.2	240.6±4.6	184.5±6.1**	142.6±6.2**	132.5±6.8**	108.5±2.7**

Biochemical Changes

Streptozotocin induced diabetic rats showed significant elevation in their blood glucose level. After the administration of BLTM at 40mg/kg b.w to the diabetic rats for 28 days, marked reduction (55.3%) in fasting blood glucose levels was noted and was comparable with the reference drug, glibenclamide at 10mg/kg b.w. The results are depicted in Table 2.

Serum cholesterol, triglycerides, SGPT, ALP and creatinine levels were elevated in diabetic rats and were significantly ($P < 0.01$)

reduced upon treatment with BLTM (40mg/kg.b.w). Serum insulin and total protein levels were decreased in streptozotocin induced diabetic rats after the treatment with BLTM, a significant ($P < 0.01$) recovery was noted and was well comparable with the reference drug. The results are shown in Table 3. Significant ($P < 0.01$) increase in glycogen content of liver & skeletal muscle was observed in BLTM treated group and it was well comparable with the standard drug. The results are shown in Figure 1.

Table 3: Effect of BLTM on biochemical parameters in streptozotocin induced diabetic rats

Group	Serum Cholesterol (mg/dl)		Triglycerides (mg/dl)		SGPT(IU/L)		ALP(IU/L)		Creatinine (mg/dl)		Insulin (μIU/ml)		Total protein (g/dl)	
	1st day	28th day	1st day	28th day	1st day	28th day	1st day	28th day	1st day	28th day	1st day	28th day	1st day	28th day
Diabetic control	145.4±4.9	144.8±4.7	167.6±3.6	169.7±4.0	108.5±1.8	110.3±1.6	339.0±6.1	365.0±6.4	1.8±0.5	1.99±0.7	9.73±0.5	9.03±0.2	4.9±0.6	4.7±0.4
Glibenclamide (10mg/kg)	148.3±5.3	83.6±4.9**	161.2±4.3	73.6±7.0**	102.1±9.4	47.6±7.5**	311.4±3.1	177.3±3.2**	1.67±0.3	1.06±0.6**	9.96±0.4	19.3±0.4**	5.0±0.14	7.03±0.5**
BLTM (40mg/kg)	136.8±5.3	73.3±4.7**	156.5±5.4	81.1±7.6**	107.0±2.3	63.1±5.2**	331.3±5.8	207.8±6.5**	1.72±0.8	0.98±0.4**	10.1±0.13	18.9±0.1**	5.1±0.17	8.15±0.3**

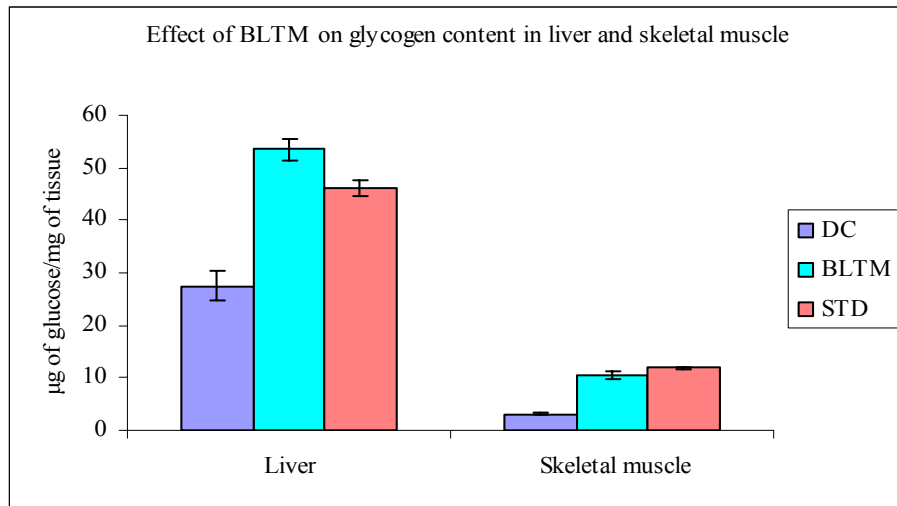


Figure.1- Effect of BLTM on glycogen content in liver and skeletal muscle

Antioxidant activity

BLTM showed better antioxidant activity by reducing the lipid peroxide levels in serum samples (4.83nmol/ml of serum), and by scavenging DPPH radical (IC₅₀:52µg/ml). The results are shown in

Table 4 and Figure 2 respectively. BLTM at 100µg/ml showed strong radical scavenging activity with percent decrease of 112% and it was comparable with standard Ascorbic acid.

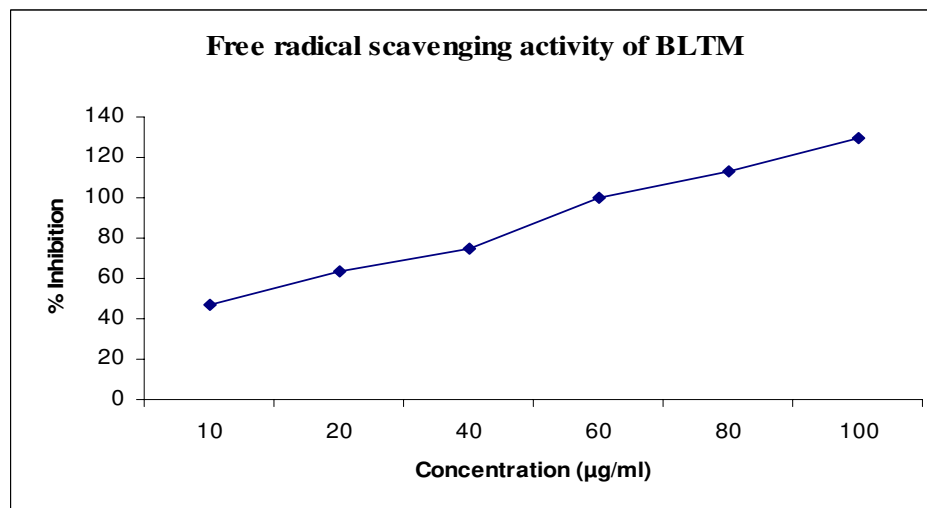


Figure.2-Free radical scavenging activity of BLTM

Table 4: Effect of BLTM on lipid peroxidation product/MDA level

Groups	Dose	MDA (nmol/ml of serum)
Normal	-	5.48 ± 0.45
Diabetic control	-	7.90 ± 0.37
Standard	10mg/Kg	5.10 ± 0.86
BLTM	40mg/Kg	4.83 ± 0.26

Histopathology of Pancreas

Figure 3 represents the histopathology of pancreas in BLTM and glibenclamide treated groups. The photo micrograph of vehicle treated normal rats showed normal acini, and normal cellular population in the pancreatic islets of langerhans. (Figure.3a).The islets of streptozotocin induced diabetic rats showed extensive damage of the pancreas, such as reducing the number of islet cells and diminishing the diameter of the islets of langerhans, so that the

islets were appeared as shrunken in case of diabetic rats compared to normal control rats (Figure.3b).The rats treated with glibenclamide showed prominent increase in size of the islets and intensity of the β cells (Figure.3c).The expansion of islet and

intensity of the β cells were greater in BLTM treated group (Figure. 3d).

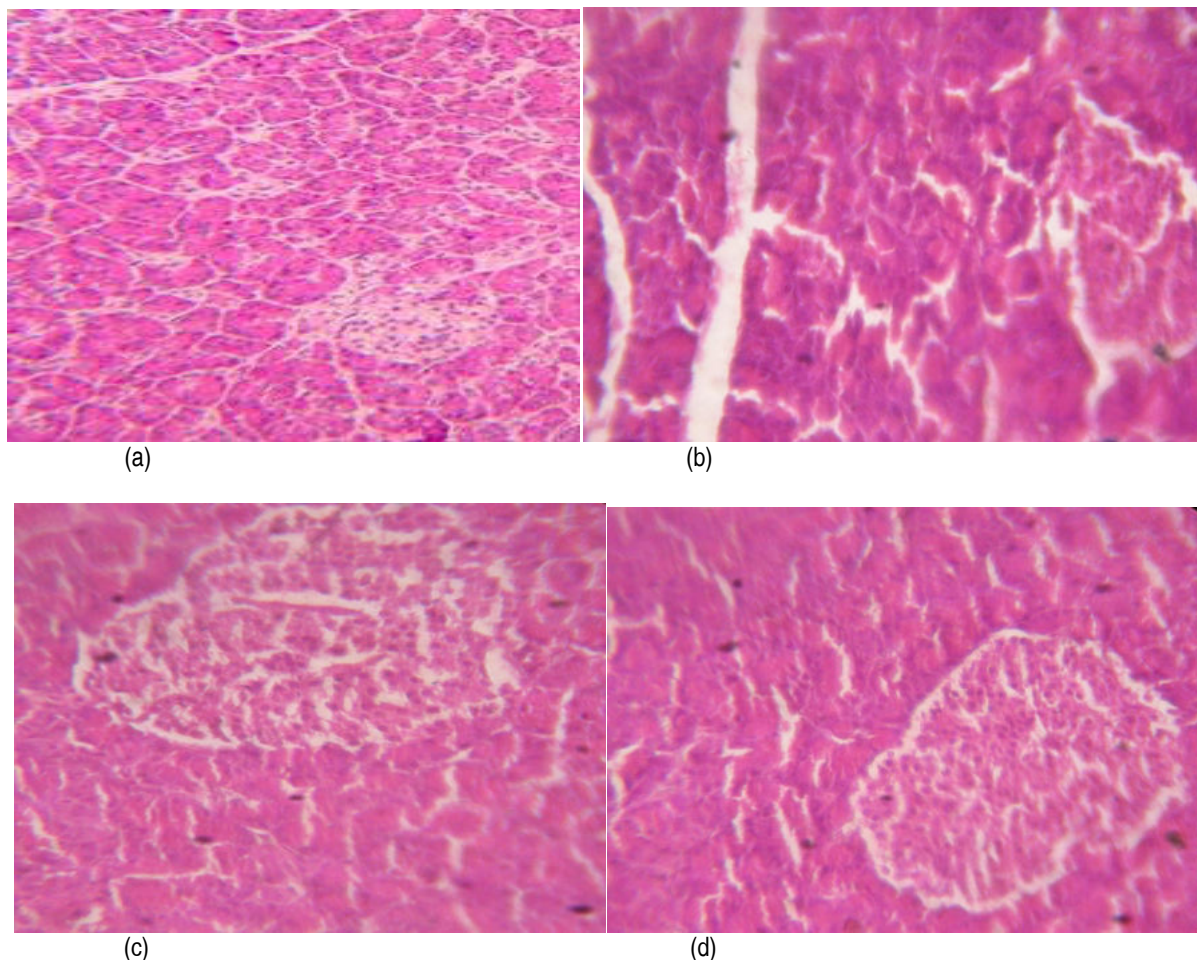


Figure.3- Histopathology of rat pancreas after 28 days of treatment with BLTM and glibenclamide.

(a). Normal control rats; (b). Diabetic rats; (c). Diabetic rats treated with glibenclamide (10mg/kg); (d). Diabetic rats treated with BLTM (40mg/kg);

HPTLC fingerprinting profile

High performance thin layer chromatogram of BLTM (Figure. 4) showed five peaks corresponding to at least five compounds.

Among the peaks, three peaks were found to be major with percentage peak area was 52.80, 18.62 and 24.20 at 0.13, 0.25 and 0.36 Rf values respectively.

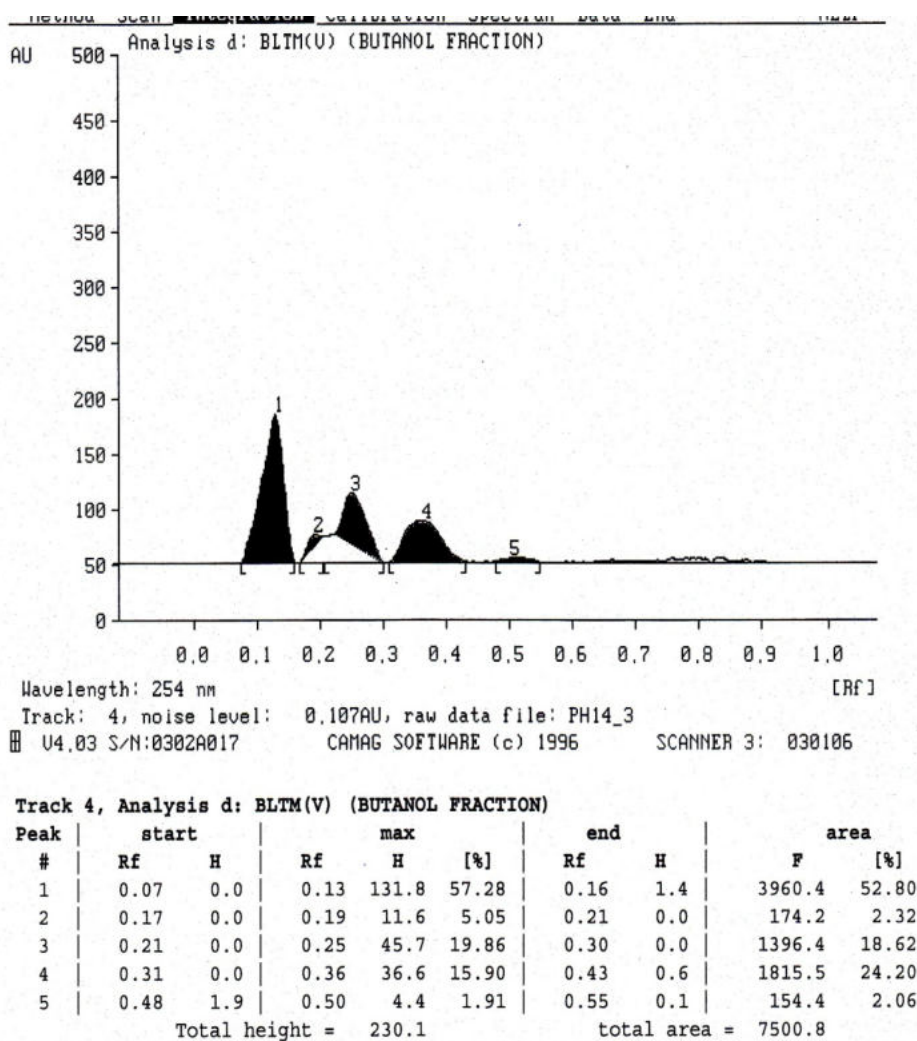


Figure.4- HPTLC finger printing profile of BLTM

Discussions

The present investigation was carried out to explore the anti-diabetic potential of whole plant of *Trichuriella monsoniae*, as it is used traditionally in the treatment of diabetes. Acute toxicity studies revealed the non toxic nature of all the three fractions (TLTM, EATM, and BLTM) of methanolic extract of *T.monsoniae*. There was no lethality (or) toxic reactions found up to the dose of 2000mg/kg and have significant ($P < 0.01$) anti-hyperglycemic activity in streptozotocin induced diabetic rats. Among the three fractions, BLTM at 40 mg/kg was found to be more effective on reducing fasting blood glucose level after 6h of administration.

In order to understand the probable mechanism(s) by which BLTM elicits its antihyperglycemic activity, various biochemical parameters were evaluated in subacute (28 days) study. Streptozotocin is a broad spectrum of antibiotic and is widely used as diabetogenic agent in experimental animals. Diabetogenic action is mediated by causing damage to the β cells in the islets of langerhans of the pancreas, resulting in a deficiency of insulin and

thereby hyperglycemia. Administration of BLTM to diabetic rats for 28 days showed a significant decrease in the elevated blood glucose and increase in the serum insulin level. This effect may be by increasing insulin secretion from the existing β cells of the pancreas or by its release from bound form. [9]

Streptozotocin induced diabetes associated with the characteristic loss of body weight, which is due to increased muscle wasting [10] and due to loss of tissue proteins [11]. BLTM treated diabetic rats showed significant ($P < 0.01$) improvement in their body weight when compared with diabetic rats, which may be due to its protective effect in controlling muscle wasting i.e. reversal of gluconeogenesis and may also be due to the improvement in insulin secretion and glycemic control. [12]. BLTM also exhibited improvement in renal function, by decreasing serum creatinine level which is generally elevated in diabetic rats.

Elevated level of hepatic enzymes (SGPT, ALP) and decreased level of total protein content in serum in diabetic rats were found to

be recovered significantly ($P < 0.01$) with the treatment of BLTM, indicating the ability of the extract to improve the liver functioning. Hypercholesterolemia and hypertriglyceridemia have been reported to occur in diabetic rats due to increase in HMGCoA reductase, a key enzyme of cholesterol biosynthesis and deactivation of the enzyme lipoprotein lipase respectively. In this study, BLTM could significantly recover the change in the lipid profile of diabetic rats. From this it may be stated that, the possible mechanism of reduction of serum lipid levels with BLTM may be through insulin release (or) by enhancing insulin sensitivity in the tissues, which was also evident from the increase in serum insulin levels, and the effect of BLTM was comparable with the standard drug glibenclamide, an insulin secretagogue.

Decreased glycogen content was observed in diabetic rats probably due to lack of insulin which results in inactivation of glycogen synthase system. The significant increase in the glycogen level in BLTM treated diabetic rats may be because of the reactivation of glycogen synthase. This focuses the one of the possible way of anti-diabetic action of BLTM by improvement of glycogenesis process [13]

During diabetes, persistent hyperglycemia causes increased production of free radicals. In the present study, involvement of free radicals in progression of disease and protective effect of BLTM has been examined by radical scavenging activity and by reducing the elevated lipid peroxidation; it was measured in the

form of nano moles of MDA/ml of the serum. As byproducts of lipid peroxidation, MDA reflects the degree of oxidation.

BLTM has profound invitro antioxidant activity as judged by DPPH radical scavenging assay and by TBARS assay.

Flavonoids, terpenoids, steroids and alkaloids are known to be bio active anti-diabetic agents [14]. Preliminary phytochemical investigations revealed the presence of flavonoidal and steroidal/terpenoidal glycosides in BLTM.

Conclusion

The butanolic fraction of methanolic extract of the whole plant of *Trichuriella monsoniae* exhibited a significant antidiabetic activity, indicating its potentiality to be a herbal antidiabetic agent. The study also substantiated the traditional claim of this plant in the treatment of diabetes.

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Conflict of Interest

The authors are declare no conflict of interest.

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