

**EVALUATION OF ANTI-DIABETIC ACTIVITY OF 2, 4, 6-
TRIHYDROXY PHENYL PROPIONATE ISOLATED FROM
TRIUMFETTA ROTUNDIFOLIA (LAM). IN STREPTOZOTOCIN
INDUCED ON WISTAR RATS**

R. Sanilkumar* and A.Kottai Muthu

Department of Pharmacy, Annamalai University, Annamalai Nagar-608002, India.

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***Correspondence for Author**

R. Sanilkumar

Department of Pharmacy,
Annamalai University,
Annamalai Nagar-608002,
India.

ABSTRACT

The anti-diabetic activity of 2, 4, 6 - Trihydroxy phenyl propionate isolated from *Triumfetta rotundifolia (Lam.)* in streptozotocin induced diabetic rats was investigated in the present study. Streptozotocin was used to induce diabetes mellitus. The anti-diabetic potential was assessed by determining oral glucose tolerance, fasting blood glucose, urine glucose, liver glycogen content, serum lipid profile, change in body weight and histopathology studies. 2, 4, 6-Trihydroxy phenyl propionate isolated from *Triumfetta rotundifolia* was administered to normal and experimental diabetic rats for 15 days. Significant ($p < 0.001$) reduction in fasting blood glucose levels was observed in the

2, 4, 6-Trihydroxy phenyl propionate is treated diabetic animals from day 7 onwards. In oral glucose tolerance test, reduction in fasting blood glucose a level was noted after 60 min of extract administration. After 15 days of treatment with extracts the maximum reduction in fasting blood glucose (53.87%) was observed in diabetic rats treated with 2, 4, 6-Trihydroxy phenyl propionate isolated from *Triumfetta rotundifolia* (50mg/kg/body wt). Serum lipid levels reversed towards near normal and the loss of body weight was controlled in treated rats as compared to diabetic control. The extract treatment also showed a significant increase in the liver glycogen content and gradual decrease in level of urine sugar level of sugar level. Microscopically examined pancreas section of rats treated with 50mg/kg isolated compound showed normal architecture of pancreas. The results demonstrate that whole plant of 2, 4, 6-Trihydroxy phenyl propionate isolated from *Triumfetta rotundifolia* possesses significant anti-diabetic activity.

Keywords: *Triumfetta rotundifolia*, 2, 4, 6-Trihydroxy phenyl propionate, Streptozotocin, Diabetes.

INTRODUCTION

Diabetes is a chronic metabolic disorder characterized by abnormalities in carbohydrate and lipid metabolism^[1], which leads to postprandial and fasting hyperglycemia, dyslipidemia and hyperinsulinemia^[2]. Although many synthetic drugs show significant therapeutic potential, their use has already been restricted due to several undesirable side effects such as hepatotoxicity, cardiomegaly and hemotoxicity^[3,4]. Despite the presence of known anti-diabetic medicines in the market, screening for new drugs from plants is still attractive because of their safety and efficacy. A number of plant species are known worldwide to have hypoglycaemic potential^[5]. *Triumfetta rotundifolia* (Lam.) is widely used in Indian traditional medicines and the leaf paste is used to treat rheumatic pain, cough, fever and severe cold^[6]. Leaf paste is taken along with pepper to treat dyspepsia^[7]. Bark paste, mixed with hot milk is used internally for treating urinary infections^[8]. *Triumfetta rotundifolia* (Lam.) is annual slender herb. Its leaves are palmately 5-lobed, scabrous along with smooth beneath, denticulate margin. Its peduncle male flowers contain calyx tube 2-4x3-6 mm, spreading lobes; greenish – yellow corolla, shortly papillose, ovate, acute; lobes. This species is widely distributed throughout India and globally distributed in tropical and subtropical region of Asia, Africa and India. The plant is being used very specifically in the indigenous systems of medicine such as Ayurveda, Siddha and Unani. However, sufficient scientific data to support these claims are still not available. Therefore, it seemed worthwhile to assess anti-diabetic potential of *Triumfetta rotundifolia*. There is no scientific evidence to support its use as anti-diabetic drug; the objective of this study was to establish the scientific basis of the use of 2,4,6-Trihydroxy phenyl propionate isolated from *Triumfetta rotundifolia* in the management of diabetes using streptozotocin- induced diabetic rats.

MATERIAL AND METHODS

Collection of Plant materials

Whole plant of *Triumfetta rotundifolia* (Lam.) was collected from Kayathar, Tuticorin District, Tamil Nadu, India. Taxonomic identification was made from botanical survey of medicinal plants, Siddha Unit, Government of India and Palayamkottai. The whole plant leaves were dried under shade, segregated, pulverized by a mechanical grinder and passed through a 40 mesh sieve.

Preparation of Extracts

The above powdered materials were successively extracted with methanol (70-80°C) for 48 hrs by continuous hot percolation method in soxhlet apparatus (Harbone J B, 1984) [9]. The extract was collected and evaporated to dryness by using a vacuum distillation unit. The dried extracts were stored in airtight container. The yield of the crude methanolic extract was found to be 16.08% w/w.

Isolation of 2, 4, 6-Trihydroxy phenyl propionate from methanolic extract of *Triumfetta rotundifolia*

The methanolic extract of whole plant of *Triumfetta rotundifolia* was subjected to column chromatographic separation using normal phase silica gel column. The dark brown solid (20g of methanolic extract of *Triumfetta rotundifolia*) was adsorbed on silica gel (20g) and transferred to a column of silica gel (200g equilibrated with benzene). 2, 4, 6- Trihydroxy phenyl propionate (193mg) was isolated with a solvent system of ethyl acetate: methanol 70:30 v/v from methanolic extract of *Triumfetta rotundifolia*.

Acute toxicity study

Acute toxicity study was performed according to OECD - 423 guidelines. Albino rats (n = 6) of either sex selected by random sampling technique were employed in this study. The animals were fasted for 4 h with free access to water only. The isolated compound of 2, 4, 6-trihydroxy phenyl propionate from *Triumfetta rotundifolia* suspended in normal saline: tween 80 (95:5) which was administered orally at a dose of 5 mg/kg initially and mortality was observed for 3 days. The mortality was observed in 5/6 or 6/6 animals, and then the dose administered was considered as toxic dose. However, the mortality was observed in less than four rats, out of six animals then the same dose was repeated again to confirm the toxic effect. The mortality was not observed, the procedure was then repeated with higher doses such as 50, 300, 1000, and 2000 mg/ kg.

Animals

Male albino wistar rats each weighing 120-150 gm was obtained from RMMCH in Annamalai University at Chidambaram (IAEC Approved Number- 767). Rodent laboratory show was access and water *ad libitum*, and rats were maintained on a 12 hour light/dark cycle in a temperature regulated room (20-25°C) during the experimental procedures. The animals were cared for according to the guiding principles in the care & use of animals.

Experimental design

All the animals were randomly divided into the four groups with six animals in each group. Group I, were administered vehicle (10ml/kg distilled water), Group II diabetic agent {streptozotocin(60mg/kg,i.p.)}+vehicle (10ml/kg,p.o.distilledwater), Group III standard {glibenclamide (10mg/kg,p.o)} and Group IV were treated with STZ (streptozotocin) + isolated compound from *Triumfetta rotundifolia*.

Drug solution

The extract was emulsified in 0.5% w/v aqueous solution of tween-80. Glibenclamide (Actavis pharmaceutical, Chennai, India) was used as a standard drug. Streptozotocin was dissolved in citrate buffer (pH4.5).

Induction of non-insulin dependent diabetes melitus(NIDDM)

NIDDM was induced in overnight fasted rats weighting 20-30g by intraperitoneal administration of streptozotocin (Sigma aldrich, Bangalore, India) solution prepared in 0.1M citrate buffer pH=4.5 at the dose of 60mg/kg body weight. Diabetes was confirmed by the determination of fasting glucose concentration on the third day post administration of streptozotocin. Blood samples were collected after 1h of administration of streptozotocin on 1st , 4th, 7th, 10th and 15th day. Elevation in blood glucose level was found to be constant throughout 15 days. Serum glucose level was determined by glucometer. Rats having serum glucose level between 300-400 mg/dl were selected for further study.

Sample collection

After completing the treatment of 2 weeks, the rats were anesthetized by diethylether and sacrificed. Blood samples were collected by cardiac puncture method and intermediately by tail vein method and blood glucose levels were estimated using Glucometer. For histopathological studies, pancreas and the liver were dissected out immediately and transferred into 10% formalin.

Oral glucose tolerance test

The oral glucose tolerance test was performed in overnight fasted (18h) normal rats. Rats divided into three groups, each consisting of six rats. Glucose (2.5g/kg, p.o) was fed 0.5h after the administration of extract. Blood samples were collected by the tail- vein method just prior to the drug administration (normal fasting) and at the time intervals of 0, 30, 60 and

120m after glucose loading. Blood glucose level was measured immediately by using glucose oxidase- preoxidase reactive strips and a glucometer.

Assessment of anti-diabetic activity of 2, 4, 6-trihydroxy phenyl propionate isolated compound from *Triumfetta rotundifolia*

Rats were made diabetic by intraperitoneal administration of streptozotocin at the dose of 60mg/kg. Treatment with plant extract was started 48h after streptozotocin injection. Blood samples were withdrawn at three day intervals till the end of study (i.e.2 weeks).

Effect of 2, 4, 6-trihydroxy phenyl propionate isolated compound from *Triumfetta Rutondifolia* on lipid profile

Blood samples were collected by the cardiac puncture method, in the centrifuge tubes and allowed to clot for 30m at room temperature. Blood samples were centrifuged at 3000rpm for 20m. Serum was separated as supernatant and stored at -20°C until analysis.

Determination of triglyceride levels

Triglyceride was estimated by method of Wako and the modifications by McGowan and Fossati method using Accurex, triglyceride determination kit. Working reagent was prepared by dissolving contents of reagent 2 enzymes (lipoprotein lipase, glycerol kinase, glycerol- 3-phosphate oxidase, peroxidase, 4-aminoantipyrine and adenosine tri phosphate (ATP) into one bottle of reagent 1 buffer (3,5 dichloro-2- hydroxybenzene sulphonate, pH7.0). it was swirled to dissolve and allowed to stand for 10m at room temperature. Serum triglycerides were hydrolyzed to glycerol and fatty acids by lipase enzyme. In the presence of ATP and glycerol-kinase, glycerol was converted into glycerol -3- phosphate and adenosine diphosphate (ADP). Glycerol -3- PO_4 oxidase dissociates glycerol-3- phosphate into dihydroxy-acetone phosphate and hydrogen peroxide. In the presence of peroxidase, hydrogen peroxide reacts with 4-aminoantipyrine to form a coloured complex. The intensity of the colour developed was proportional to the triglycerides concentration and was adjusted to zero with distilled water. 10 μl sample was taken with the help of pipette into a cuvette. It was mixed and incubated for 5m. at 37°C . The absorbance of the samples and standard of 200mg/dl concentration was read out against the blank. The colour should be stable for 30m. Triglyceride was estimated by using the following formula

Triglyceride (Mg/dl) = (Absorbance of Samples/ Absorbance of standard) x conc. of standrad (mg/dl)

Estimation of total serum cholesterol (STC)

Total cholesterol was estimated by CHOD-POD enzymatic colorimetric end point^[10] method using accurex, cholesterol determination kit. For this method, 0.01 ml each of serum as a test, standard sample and distilled water as blank along with 1.00ml reaction solution were pipette into reaction vessels using a micropipette.

Estimation of serum lipoprotein

Phosphotungstate method^[11] was used to estimate the serum lipids like very low density lipoprotein (VLDL), low density lipoprotein (LDL) and high density lipoprotein (HDL) cholesterol level. The clear supernatant after removal of VLDL and LDL, containing HDL was used for determination of HDL- cholesterol (HDLc). The VLDL and LDL from serum were precipitated by phosphotungstate in the presence of magnesium ions.

Serum / plasma phosphotungstate, mg^{2*} HDL+ (LDL+ VLDL+ Chylomicrons)
 $\xrightarrow{\hspace{10em}}$
 VLDL- cholesterol (VLDLc) and LDL- cholesterol (LDLc) were respectively calculated by using Fredreickson-Friedwald's^[12] formula as follows

$$\text{LDL Cholesterol} = \text{Total Cholesterol} - \frac{\text{Triglyceride}}{5} - \text{HDL Cholesterol}$$

$$\text{VDDL Cholesterol} - \frac{\text{Triglyceride}}{5}$$

For this 0.5ml of serum was taken in a test tube and 0.5ml of precipitation reagent was added. The mixture was shaken thoroughly and left to stand for 10m at 25 to 30⁰c and then centrifuged for 20m at 4000rpm. Within 2h after centrifugation, the clear supernatant was used for the determination of HDL-Cholesterol. The supernatant containing 0.05ml was taken in a test tube and 1ml reaction solution was added to it. In another test tube, 0.1ml distilled water was taken and 1ml reaction solution was added. The mixtures were mixed thoroughly, incubated for 5m at 37⁰c and measured for the absorbance of the sample against blank reagent at 510nm in Biochemistry Auto analyzer (STAR 21plus, RAPID diagnostic Pvt.Ltd).

Change in body weight Body weight was taken before and after experiment at the intervals of 1st, 4th, 7th, 10th and 15th day of study with the help of single pan balance. The change in the body weight was noted.

Urine glucose estimation

The urine glucose levels were estimated by “Benedict’s test for glucose as reducing sugar in urine method”. The urine was collected from the STZ –induced diabetic rats individually in a clean beaker on the day 1,4,7,10 and 15 and the glucose level was determined.

Liver glycogen estimation

The determination of glycogen in liver was done by solution of “anthrone reagent ^[13]”. Purified anthrone (500mg), thiourea (10g) and 1 liter of 72% sulfuric acid were placed in a flask. The mixture was heated up to 80-90⁰c. The flask was occasionally shaken to mix the contents. The mixture was cooled and stored in a refrigerator. Stock solution of standard was prepared by dissolving 100mg of dry glucose in 100ml of saturated benzoic acid solution. 5ml stock solution was placed in a 100ml volumetric flask and the volume was made up with saturated benzoic acid solution.

Liver was blended by blender under trichloroacetic acid (TCA) and homogenized for 3m. The homogenate was poured into a centrifuge tube. The supernatant fluid was poured into a centrifuge tube. The supernatant fluid was centrifuged and decanted upon an acid- washed filter paper placed in a funnel and drained into a graduated cylinder. The residue was quantitatively transferred to the blender with TCA and homogenized again for 1m. The mixture was centrifuged and the supernatant fluid was poured through the same filter. Two more extractions were made in the same manner. The desired volume was made up with 5 percent TCA and the solution was mixed thoroughly. 1ml of the TCA filtrate was pipetted into a 15ml Pyrex centrifuge tube. Duplicate samples of each unknown were analyzed to obtain the most reliable results. To each tube, 5 volumes of 95 percent ethanol were added with careful blowing. This was checked by noting the absence of an interface. The tubes were capped with clean rubber stoppers and allowed to stand overnight at room temperature. After precipitation was completed, the tubes were centrifuged at 3000r.p.m. for 15m. The liquid was gently decanted from the packed glycogen and the tubes were allowed to drain in an inverted position for 10m.

The glycogen was dissolved by adding 2ml of distilled water, the water being added in a manner that was washed down the sides of the tube. Blank reagent was prepared by pipetting 2ml of water into a clean centrifuge tube. A standard was prepared by pipetting 2 ml of standard glucose solution, containing 0.1mg of glucose, into a similar tube. At this point 10ml of anthrone reagent was delivered into the centre of the each tube with vigorous, but

consistent, blowing to ensure good mixing. As each tube received anthrone reagent, it was tightly capped with an air condenser and placed in a cold tap water bath. After the temperature of all tubes had reached the temperature of cold water, they were immersed in a boiling water bath to a depth a little above the level of the liquid in the tubes for 15 m and then removed from water bath and cooled to room temperature. The tubes and stoppers were wiped dry and the contents of each tube were transferred to a calorimeter tube and the absorbance was read at 620nm after adjusting the calorimeter with the blank reagent. Care was taken to avoid introduction of lint or contaminating carbohydrate into the anthrone reaction. The calculation of glycogen content was done by using the following formula^[14].

$$\frac{\text{DU}}{\text{DS}} \times 0.1 \times \frac{\text{Volume of Extract}}{100 \text{ gm. Of Tissue}} \times 100 \times 0.9 = \text{mg.of glycogen per 100 gm.Of tissue}$$

Where DU= Optical density of the unknown, DS= Optical density of the standard, 0.1= mg of glucose in 2ml of standard solution, 0.9= factor for converting glucose value to glycogen value.

Histopathological studies

Isolated pancreas was preserved in 10% formalin for 24h. Pancreas was fixed in Bouin's fluid and cut in section of 3-5 μ m thickness and stained by hematoxyline- eosin stain. The photomicrographs of each tissue section were taken using electron microscope.

Statistical analysis

Values are presented as mean \pm standard deviation for groups of six animals. The results were analyzed by one way analysis of variance (ANOVA) followed by post hoc Dunnett's multiple comparison test. Differences between means were considered to be statistically significant at ($p \leq 0.05$).

RESULTS

Acute toxicity study

Acute toxicity study showed that 2,4,6-trihydroxy phenyl propionate isolated from *Triumfetta rotundifolia* did not produce any toxic symptoms when administered orally to rats. The lethal dose (LD-50 value) was of 2000 mg/kg body weight. Hence therapeutic dose was calculated as 1/10th (200mg/kg body weight) of the lethal dose for the antidiabetic investigation.

Fasting blood glucose determination

The effect of treatment of the extract on fasting blood glucose levels is depicted in Table no.1. Glibenclamide (GBC) treated diabetic rats of standard group III showed significant reduction in blood glucose values on day 1, 4,7,10 and 15 respectively in comparison to diabetic control groupII. This indicated that the GBC treatment successfully reduced the blood glucose levels in the diabetic rats towards the normal level in 15 days. Similarly, 2, 4, 6-trihydroxy phenyl propionate isolated from *Triumfetta rotundifolia* treated diabetic group IV showed significant reduction in blood glucose values on day 1, 4,7,10 and 15 respectively as compared with diabetic control group II. This indicated that the 2,4,6-trihydroxy phenyl propionate isolated from *Triumfetta rotundifolia* treatment could reduce the blood glucose levels in the diabetic rats towards the normal level in the 15 days of study.

Biochemical parameters

Oral glucose tolerance test (OGTT) in normal rats

Treatment with GBC significantly improved the glucose tolerance at normal fasting levels at 0, 30, 60 and 120m, respectively. Further, treatment with 2, 4, 6-trihydroxy phenyl propionate isolated from *Triumfetta rotundifolia* significantly reduced sugar glucose level at 120m compared to normal control. These data suggested that treatment with 2, 4, 6-trihydroxy phenyl propionate isolated from *Triumfetta rotundifolia* showed tolerance to glucose administration. (Table No.2).

Urine glucose estimation

The urine sugar levels in normal and diabetic group of rats are given in Table no.3. The normal control rats showed absence of sugar in urine. The urine sugar levels of the differentiate groups of diabetic animals treated with standard drug (Glibenclamide) and 2,4,6-trihydroxy phenyl propionate isolated from *Triumfetta rotundifolia* for 15 days decreased towards the normal level.

Estimation of liver glycogen content

There was significant increase in liver glycogen level to 472.24 ± 63.91 ($p < 0.001$) on day 15 in glibenclamide treated diabetic control group III. Similarly 2, 4, 6-trihydroxy phenyl propionate isolated from *Triumfetta rotundifolia* treatment significantly ($p < 0.001$) increased the glycogen content to 382.84 ± 41.95 ($p < 0.001$) in STZ-induced diabetic groupIV (Table No.4).

Estimation of lipid profile parameters

2, 4, 6-trihydroxy phenyl propionate isolated from *Triumfetta rotundifolia* exhibited significant reduction ($p < 0.001$) in all tested lipid parameters. A marked increase in total cholesterol and decrease in HDLc were observed in untreated diabetic rats. 2, 4, 6-trihydroxy phenyl propionate isolated from *Triumfetta rotundifolia* administration decreased serum triglycerids (STG); total cholesterol (STC), LDL and VLDL levels and increased HDLc level. The markers of dyslipidemia such as STC/ HDLc and LDLc/ HDLc ratios were found to be significantly elevated in the diabetic group (Table No.5).

Change in body weight

The body weight was slightly increased (148 ± 1.21 g) in the normal control group I as compared to initial body weight. Whereas in diabetic control group II, there was marked decrease (119 ± 0.5 g) in the body weight. Group III treated gilbenclamide and the group IV pretreated with 2, 4, 6-trihydroxy phenyl propionate isolated from *Triumfetta rotundifolia* increased body weight significantly to 122 ± 1 g ($p < 0.01$) and 112 ± 0.57 g ($p < 0.05$) respectively. Although there was a marginal reduction in the body weight of animals in these groups, compared to the final weight of normal control rats (Table No.6).

Table 1: Effect of 2,4,6-Trihydroxy phenyl propionate from *Triumfetta rotundifolia* on fasting blood glucose level in diabetic rats.

Groups	Treatment	Fasting blood glucose level (mg/dl)				
		Day 1	Day 4	Day 7	Day 10	Day 15
I	Distilledwater (10ml/kg, p.o)	119.5±11.5	112.15 ± 8.9	106 ± 14.53	115.79± 15.26	117.79 ± 13.0
II	STZ(60mg/kg,i.p)+ distilledwater (10ml/kg,p.o)	311.57± 33.5	324.15± 39.83	325.4 ± 36.47	307.5 ± 50.86	304.5 ± 46
III	STZ (60mg/kg,i.p)+ GBC (10ml/kg,p.o)	297.5±34.55**	214.1± 29.96***	167.17± 39.65***	149.5± 26.04***	128.32± 18.21 ***
IV	STZ(60mg/kg,i.p)+isolated compound from <i>Triumfetta rotundifolia</i> (50mg/kg,p.o)	300.57± 27.72**	263.17±26.20**	201.17± 28.52***	170.54±16.60***	139.66± 20.79***

*** p< 0.001 vs. diabetic control group II, **p< 0.01 vs. diabetic control group II

Table 2: Effect of 2,4,6-Trihydroxy phenyl propionate from *Triumfetta rotundifolia* on OGTT in normal rat

Groups	Treatments	values (mg/dl) 120m	Blood glucose concentration (mg/dl) Normal fasting			
			120m	0m	30m	60m
I	Glucose (2.5g/kg)	123.65 ± 4.9	268.82 ± 3.42	372.65 ± 4.07	249.32 ± 4.4	173.5 ± 4.4
II	GBC (10mg/kg)	103.82 ± 2.31***	180.82± 5.06***	236.5 ± 2.72***	164.15± 3.31***	146.1 ± 2.82***
III	Isolated compound from <i>Triumfetta rotundifolia</i> (50mg/kg)	107.66 ± 4.83***	189.3 ± 4.12***	265.66 ± 4.54***	185.4 ± 5.42***	155.16 ± 8.30***

*** p< 0.001 vs.normal control, OGTT=oral glucose tolearance test, GBC=glibenclamide

Table 3: Effect of 2,4,6-Trihydroxy phenyl propionate from *Triumfetta rotundifolia* on urine glucose level in STZ – induced diabetic rats

Groups	Treatment	Intensity of glucose in urine (colour change of the precipitate)				
		1(days)	4(days)	7(days)	10(days)	15(days)
I	Distilledwater(10ml/kg, p.o)	Nil	Nil	Nil	Nil	Nil
II	STZ (60mg/kg,i.p)+ distilledwater (10ml/kg,p.o)	+++	+++	+++	+++	++++
III	STZ(60mg/kg,i.p+GBC (10ml/kg,p.o)	+++	++	++	+	+
IV	STZ(60mg/kg,i.p)+isolated compound from <i>Triumfetta rotundifolia</i> (50mg/kg,p.o)	+++	+++	++	++	+
Keys:(+) = mild, (++) = moderate, (+++) = higher , (++++)=severe , STZ= streptozotocine, GBC =glibenclamide						

Table 4: Effect of 2,4,6-Trihydroxy phenyl propionate from *Triumfetta rotundifolia* on liver glycogen content in STZ – induced diabetic rats.

Groups	Treatment	Liver glycogen content on day 15 (mg/100gm)
I	Distilledwater(10ml/kg, p.o)	541.85 ± 31.63
II	STZ(60mg/kg,i.p)+distilledwater (10ml/kg,p.o)	262.94± 8.61
III	STZ(60mg/kg,i.p +GBC (10ml/kg,p.o)	472.24 ± 63.91 ^{***}
IV	STZ(60mg/kg,i.p)+isolated compound from <i>Triumfetta rotundifolia</i> (50mg/kg,p.o)	382.84 ± 41.95 ^{***}
*** p< 0.001 compared to diabetic control, STZ= streptozotocine, GBC =glibenclamide		

Table 5: Effect of 2,4,6-Trihydroxy phenyl propionate from *Triumfetta rotundifolia* on lipid profile in STZ – induced diabetic rats

Treatment Groups	STG(mg/dl)	STC(mg/dl)	HDLc(mg/dl)	LDLc(mg/dl)	VLDLc(mg/dl)	STC/HDLc ratio	LDLc/HDLc ratio
I Normal control	89.66±4.4	81.32±3.78	37.86 ± 1.01	24.32± 2.26	17.12 ± 0.89	1.05±0.05	0.66± 0.01
II diabetic control	131.66±4.15	156.2±5.28	17.66 ± 1.51	110.7 ± 6.5	27.52 ± 0.82	7.43±0.88	5.02± 0.7
III Standard	123.5±1.98*	129.36±5.49*	27.5±3.0**	71.2±3.8**	19.3±0.47*	1.89±0.18**	1.5±0.1**
IV isolated compound from <i>Triumfetta rotundifolia</i> (50mg/kg)	116±2.64*	132.32±5.51*	32.66±3.05**	75.26 ± 4.0**	22.3 ± 0.52*	2.98±0.22**	2.23± 0.18**

*p< 0.05, **p<0.01 compared to diabetic control, STZ=streptozotocine, GMC=glibenclamide, STG= total serum triglyceride, STC= total serum cholesterol, HDLc= high density lipoprotein cholesterol, LDLc= low density lipoprotein cholesterol.

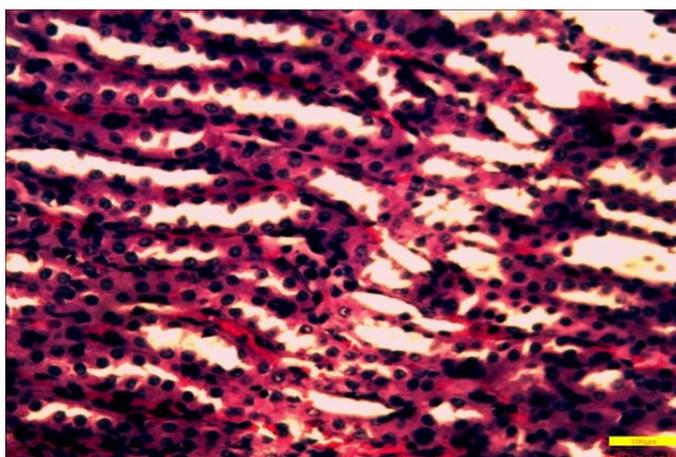
Table 6: Effect of 2,4,6-Trihydroxy phenyl propionate from *Triumfetta rotundifolia* on body weight(gm) in STZ – induced diabetic rats.

Groups	Treatment	Body weight(s)	
		Day 1	Day 15
I Normal control	Distilledwater(10ml/kg, p.o)	145±0.97	148±1.21
II Diabetic control	STZ (60mg/kg,i.p)+ distilledwater (10ml/kg,p.o)	129±0.65	119±0.5
III Standard	STZ(60mg/kg,i.p)+GBC (10ml/kg,p.o)	124±0.85	122±1**
IV isolated compound from <i>Triumfetta rotundifolia</i>	STZ(60mg/kg,i.p)+isolated compound from <i>Triumfetta rotundifolia</i> (50mg/kg,p.o)	114±0.92	112±0.57*

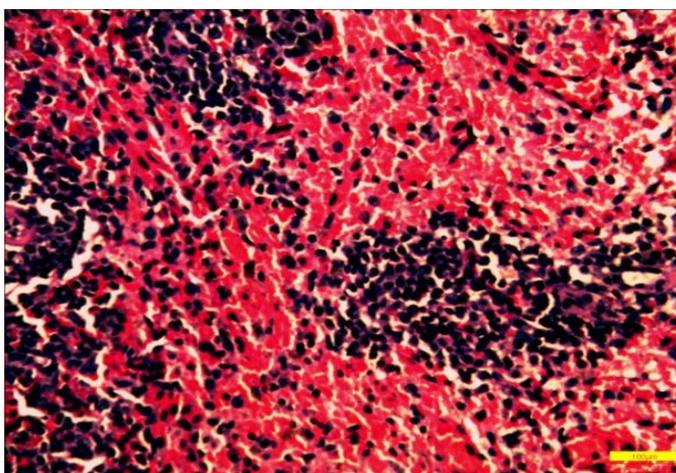
**p<0.01, *p<0.05 compared to diabetic control, STZ= streptozotocine, GBC= glibenclamide

Histopathology

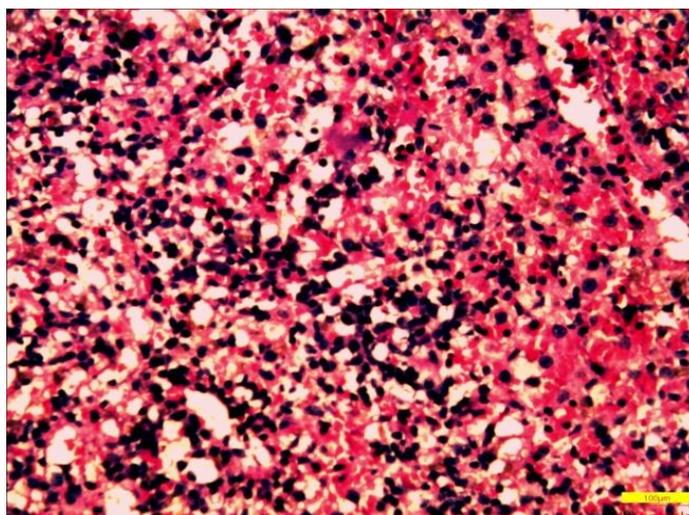
Photomicrographs showed normal architecture of pancreas with acini of serous epithelial cells along with nest of endocrine cells separated by fibrocollagenous, stroma into lobules of vehicle- treated rats (Fig.1a). Extensive damage to the acini of serous epithelial cells and islets of langerhans(Fig.1b), restoration of normal architecture of pancreas with acini of serous epithelial cells by glibenclamide(Fig.1c) are also shown. The partial restoration of normal cellular population and normal architecture of pancreas with acini of serous epithelial cells along with nest of endocrine cells separated by fibrocollagenous, stroma into lobules was shown by methanol extract. No fibrosis or inflammation was noted (Fig.1d).



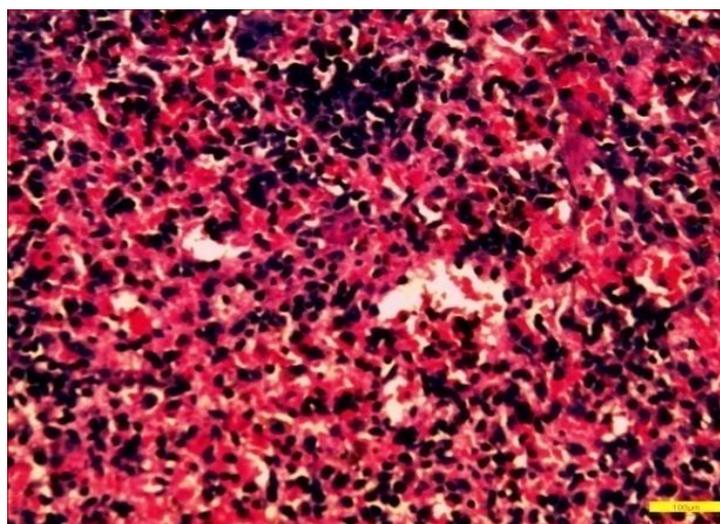
a. Control:Distilled water (10ml/kg,p.o)



b. Diabetic control : STZ (60mg/kg, i.p.)



c. Standard : GBC (10mg/ kg, P.O)



d. Treated: Isolated compound from *Triumfetta rotundifolia* (50mg/kg,p.o)

DISCUSSION

The aim of present study was to investigate the anti-diabetic potential of 2,4,6-trihydroxy phenyl propionate isolated from *Triumfetta rotundifolia*, using STZ-induced diabetic rats model. Hyperglycaemia produced by STZ exhibited marked increase in serum triglycerids and total cholesterol. Under normal conditions, the enzyme lipoprotein lipase hydrolyses triglycerides. Diabetes mellitus results in failure to activate this enzyme thereby causing hypertriglyceridemia. Elevated serum total cholesterol, triglycerides and decreased high density lipoprotein level were observed in diabetic control rats. Chronic administration of the extract for 15 days to the STZ-induced diabetic rats significantly ($p < 0.05$) produced a fall in blood glucose level and lipid profile. Hence the isolated compound of *Triumfetta rotundifolia* may be considered to have good anti hyperglycemic activity and did not cause any hypoglycemic effect unlike insulin and other synthetic drugs. Normalization of the blood

glucose level resulted in significant reduction in the level of serum cholesterol and triglycerides. The antihyperglycemic activity caused by glibenclamide and isolated compound from *Triumfetta rotundifolia* in streptozotocin induced diabetic rats indicates normalization of serum lipid profile and stimulation of insulin secretion from beta cells. The observed hypolipidaemic effect may be because of decreased cholesterologenesis and fatty acid synthesis. Significant lowering of total cholesterol and elevation of HDL cholesterol are very desirable biochemical states for prevention of atherosclerosis and ischemic conditions.

In diabetic control group, the characteristic loss of body weight is caused by an increase in muscle wasting and loss of tissue proteins^[15]. The difference in the body weight observed during the period of treatment of the rats treated with isolated compound of *Triumfetta rotundifolia* was less as compared to the diabetic control group, which may be due to its protective effect in controlling muscle wasting, i.e. reversal of gluconeogenesis and may also be due to proper glycemic control. Based upon these results it can be hypothesized that isolated compound from *Triumfetta rotundifolia* probably acting by releasing insulin from pancreatic β cells. The hypothesis is further supported by the pancreatic histology which showed protection of pancreatic β cells from toxic effect of STZ. The difference observed between the initial and final fasting blood glucose levels of different groups under investigation revealed a significant elevation in blood glucose in diabetic control group at the end of the 15th day experimental period. When isolated compound from *Triumfetta rotundifolia* was administered to glucose loaded normal rats fasted for 18 h, reduction in blood glucose levels was observed after 60m. The decline in the level blood glucose reached its maximum at 120m. Administration of the extract to diabetic rats showed a significant decrease in the fasting blood glucose. Hence, the possible mechanism of anti-hyperglycemic action of isolated compound from *Triumfetta rotundifolia* is the potentiation of the insulin effects of plasma by increasing either the pancreatic secretion of insulin from the existing beta cells or by its release from the bound form.

The decrease in hepatic glycogen content in diabetes is probably due to lack of insulin in the diabetic state which results in the inactivation of glycogen synthase enzyme^[16]. The significant increase in the glycogen content of the treated groups may be because of reactivation of the glycogen synthase enzyme. Hence, improvement of glycogenesis may be another probable way of anti-diabetic action^[17]. The anti-hyperglycemic activity caused by glibenclamide and isolated compound from *Triumfetta rotundifolia* in streptozotocin-induced

diabetic rats indicates normalization of serum lipid and stimulation of insulin secretion from beta cells. Flavonoids, sterols/triterpenoids, alkaloids and phenolic compounds are known to be bioactive anti-diabetic principles^[18]. Flavonoids are known to regenerate the damaged beta cells in the alloxan-induced diabetes in rats^[19]. Phenolic compounds are found to be effective anti-hyperglycemic agents^[20]. The anti-diabetic effect of isolated compound from *Triumfetta rotundifolia* may be due to the presence of more than one anti-hyperglycemic constituent and their synergistic properties.

CONCLUSION

It is thus concluded that 2, 4, 6-trihydroxy phenyl propionate isolated from *Triumfetta rotundifolia* has promising anti-diabetic effect, which potentially improved abnormalities of diabetic conditions in streptozotocin induced diabetic rats. The probable hypoglycemic effect of 2, 4, 6-trihydroxy phenyl propionate isolated from *Triumfetta rotundifolia* may be attributed to increase in serum and pancreatic insulin levels. However, longer duration studies on chronic models are required to elucidate the exact anti-diabetic mechanism of action. As well as there is a need to isolate bioactive principles which can be developed as potent anti-diabetic drug.

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