G.Gopalakrishnan et al /J. Pharm. Sci. & Res. Vol. 6(2), 2014, 97-103

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Evaluation of Anti-diabetic Activity of Methanolic Extract of *Coleus vettiveroides* Jacob in Streptozotocin-induced Diabetic rats.

G.Gopalakrishnan , C.K.Dhanapal
Department of Pharmacy,
Faculty of Engineering & Technology, Annamalai University,
Annamalai Nagar-608002, Tamil Nadu, India.

Abstract

Objective: *The aim of the study was to evaluate the anti-diabetic activity of methanolic extract of Coleus Vettiveroides Jacob in streptozotocin induced diabetic rats.*

Methods: *Streptozotocin was used to induce diabetes mellitus. The anti-diabetic potential was assessed by determining oral glucose tolerance, fasting blood glucose, urine glucose estimation, changes in body weight, Liver functions (SGPT and ALP), kidney functions (Urea and Creatinine), Pancreatic functions (Liver Glycogen and Serum Amylase) and Serum (Total Bilirubin, Total Protein, Total Cholesterol, Triglycerides) and histopathological studies was done for the control and experimental rats.*

Results: *Methanolic extract of coleus vettiveroides jacob was administered to normal and experimental diabetic rats for 15 days. Significant reduction in fasting blood glucose levels was observed in the methanolic extract 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 was observed in diabetic rats treated with methanolic extract of Coleus Vettiveroides Jacob (200mg/kg & 400mg/kg body wt) and the loss of body weight was controlled in treated rats as compared to diabetic control. The extract treatment also showed a significant decrease in level of urine sugar level of sugar level. Streptozotocin treatment leads to elevated levels of Serum Amylase, Triglycerides, Total Cholesterol, SGPT, ALP, Bilirubin, Blood Urea and Creatinine and decreased the levels of Liver Glycogen and Total Protein. However, treatment with methanolic extract of coleus vettiveroides Jacob significantly reversed the above changes compared to the control group as observed in the streptozotocin-induced rats. Microscopically examined pancreas section of rats treated with methanolic extract showed normal architecture of pancreas.*

Conclusion: *The results demonstrate that methanolic extract of Coleus Vettiveroides Jacob possesses significant anti-diabetic activity.*

Keywords: **Coleus Vettiveroides Jacob, Streptozotocin, Serum parameters**

INTRODUCTION

Diabetes mellitus is considered the commonest endocrine disorder and it is the sixth leading cause of death globally[5]. Increase in blood glucose damages many of the body's systems, in particular, the blood vessels and nerves. The hyperglycemia caused due to decreased insulin production is called Type-1 diabetes and hyperglycemia due insufficient insulin utilization is called Type-2 diabetes[1]. It is estimated that diabetes in adults is over 170 million worldwide and its prevalence is likely to increase to over 300 million by the year 2025 [2, 3].

Since ancient times, plants have been an exemplary source of medicine. Ayurveda and other Indian literature mention the use of plants in treatment of various human ailments. India has about 45000 plant species and among them, several thousands have been claimed to possess medicinal properties [4]. The commonly practiced treatment of diabetes includes oral antidiabetic drugs, insulin injection and management through diet and physical exercise. Apart from currently available therapeutic for the treatment of diabetes, traditional plant medicines are also used throughout the world for treatment of diabetes. The use of these plants is based on the belief that they have low toxicity and cost less than the semi-synthetics or synthetics.

Coleus vettiveroides Jacob (Lamiaceae) is a small profusely branched succulent herb with quandragular stems and branches and deep straw coloured aromatic roots .Leaves are glandular and hairy, broadly ovate with dentate margins and prominent veins in the abaxial side. The whole plant is used in ayurvedic system of medicine for treating varied diseases like leprosy, skin diseases, leucoderma, fever etc [6]. The formulations are mostly for internal use, and generally indicate a therapeutic activity in cases of G.I disorders like mal-absorption, flatulence, diarrhoea or dysentery and ulcers resulting from such G.I related syndromes. Externally, the Taila and Lepa formulations are used as emollients and plasters over painful areas. The other drugs in such formulations along with which Hrivera is usually added are generally plant drugs containing essential oils with known carminative and analgesic properties. The Present study was taken up to evaluate the antidiabetic activity of *Coleus vettiveroides Jacob* and to establish its therapeutic potential in the treatment of diabetes and its complications.

MATERIALS AND MET HODS

Preparation of the Extract

The entire plant of *Coleus vettiveroides Jacob* was collected from Tirunelveli district, Tamilnadu, India. Taxonomic identification was made from botanical survey

of medicinal plants, Siddha Unit, Government of India and Palayamkottai. The dried powdered plant material was extracted with methanol for 72 hours by using soxhlet apparatus[7]. The extract was filtered and concentrated to dryness in vacuum and stored in an air tight container.

Animals

Wistar albino rats (150- 200g) was obtained from RMMCH in Annamalai University at Chidambaram (IAEC Proposal Number- 794) were used for the study. The animals were fed with commercial pellets and water ad libitum. The animals were well acclimatized to the standard environmental conditions of temperature ($22^oC \pm 5^oC$) and humidity (55 ± 5^oC) and 12 hrs light/dark cycle throughout the experimental period.

Acute Toxicity Studies

The acute oral toxicity study was carried out as per OECD 423 guidelines (OECD, 2001). The study was approved by the Institutional Animal Ethics Commitee (IAEC). No mortality and no signs of toxicity were found even after administration of a limit dose of 200 mg/kg body weight of extract; hence $1/10^{th}$ of the dose was taken as effective dose. Two doses, 200 and 400mg/kg were selected for the present study to evaluate anti-diabetic activity.

Experimental Protocol

The animals were divided into five groups of six animals each. Group I served as normal control treated with normal saline in a dose of 10ml/kg, group II served as a toxic group and was administered Streptozotocin 50mg/kg body weight, Group III served as as a treatment control group and was administered methanolic extract of *Coleus vettiveroides Jacob* at the dose of 200 mg/kg body weight, Group IV a treatment control group and was administered methanolic extract of *Coleus vettiveroides Jacob* at the dose of 400 mg/kg body weight and GroupV served as a standard group and was administered Glibenclamide 10mg/kg body weight.The extract was emulsified in 0.5% w/v aqueous solution of tween-80. Gilbenclamide (Actavis pharmaceutical, Chennai, India) was used as a standard drug. Streptozotocin was dissolved in citrate buffer (pH4.5). Body weight was taken before and after experiment at the intervals of 1^{st} and 15^{th} day of study with the help of single pan balance. The change in the body weight was noted.

Induction of Diabetes

Diabetes 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 50mg/kg body weight. Diabetes was confirmed by the determination of fasting glucose concentration on the post administration of streptozotocin. Blood samples were collected after 1hour of administration of streptozotocin. 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. The changes of body weight was observed throughout treatment period in experimental animals.

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 animals were divided into four groups of six rats in each group.

Group-I: Glucose (2 g/kg p.o. b.w.), Group-II: MECVJ (200 mg/kg) and glucose (2 g/kg p.o. b.w.) , Group-III: MECVJ (400 mg/kg) and glucose (2 g/kg p.o. b.w.) and Group-IV: Glibenclamide (10 mg/kg) and glucose (2 g/kg p.o. b.w.). The animals were fasted overnight and treated with above dosage schedule orally. The MECVJ and glibenclamide were administered half an hour before administration of glucose solution. 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, 60,120 and 240m after glucose loading. Blood glucose level was measured immediately by using glucose oxidase- preoxidase reactive strips and a glucometer.

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.

BIOCHEMICAL ANALYSIS

Dissection and Homogenization

After 14 days of treatment all the rats were sacrificed by cervical decapitation and the blood was collected by the cardiac puncture, in the centrifuge tubes and allowed to clot for 30m at room temperature. Blood samples were centrifuged at 3000 rpm for 20 minutes. Serum was separated as supernatant and stored at -20^0c until analysis.

Biochemical Estimation

Triglyceride was estimated by method of Wako and the modifications by McGowan and Fossati method using Accurex, triglyceride determination kit, Total cholesterol was estimated by CHOD-POD enzymatic colorimetric end point[16],Total protein (TP) levels were determined by the method of Lowry *et al*.,(1951)[17], Liver glycogen was determined according to the method of Hassid and Abraham and modified by Good et al.,(1975)[18], and Serum α-Amylase was determined by the method of Street et al.,(1956)[22], Serum ALP was measured according to the method of Kind and King(1954)[19] , and SGOT were measured according to the method of Rietman and Frankel[20], The Toro et al., (1975)[23] method was used to evaluate the Serum Creatinine levels, and Serum Bilirubin activity was determined according to the method of Malloy and Evelyn (1937)[21].Serum Urea was determined according to the method of Marsh et al., as described in the textbook of clinical biochemistry, Varley et al.,(1980).

Histopathological studies

Isolated pancreas was preserved in 10% formalin for 24 hours. Pancreas was fixed in Bovine's fluid and cut in

section of 3-5µm 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 ± standard deviation for groups of six animals. The differences between control and treatment groups were tested for significance using ANOVA followed by Dunnet's t test. $P<0.05$ were considered significant.

RESULTS

Acute toxicity study

Acute toxicity study showed that methanolic extract of *Coleus Vettiveroides Jacob* 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 $1/10^{th}$(200mg/kg & 400mg/kg body weight) of the lethal dose for the antidiabetic investigation.

Changes in body weight

Table 1 showed that the effect of methanolic extract of *Coleus Vettiveroides Jacob* on body weight in streptozotocin induced wistar rats. The body weight was slightly increased (233.41±1.73g) in the normal control group I as compared to initial body weight. Whereas in diabetic control group II, there was marked decrease (171.34±3.21g) in the body weight. Group V treated with gilbenclamide and the Group III& IV pretreated with MECVJ increased body weight significantly to 239.29±1.88g and 227.21±1.93 & 236.75±1.45g 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.

Fasting blood glucose determination

The effect of treatment of the extract on fasting blood glucose levels is depicted in Table no.2. Glibenclamide (GBC) treated diabetic rats of standard group V showed significant reduction in blood glucose values on day 1, 7 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, MECVJ treated diabetic group III & IVshowed significant reduction in blood glucose values on day 1, 7and 15 respectively as compared with diabetic control group II. This indicated that the MECVJ treatment could reduce the blood glucose levels in the diabetic rats towards the normal level in the 15 days of study.

Oral glucose tolerance test (OGTT) in normal rats:

Table 3 represents the effect of methanolic extract of *Coleus Vettiveroides Jacob* on Oral glucose tolerance test (OGTT) in normal rats.Treatment with GBC significantly improved the glucose tolerance at normal fasting levels at 0, 30, 60, 120 and 240m, respectively. Further, treatment with MECVJ significantly reduced sugar glucose level at 240m compared to normal control. These data suggested that treatment with MECVJ showed tolerance to glucose administration.

Urine glucose estimation:

Table 4 showed that the effect of methanolic extract of *Coleus Vettiveroides Jacob* on 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 MECVJ for 15 days decreased towards the normal level.

BIOCHEMICAL PARAMETERS

Effect Of Coleus Vettiveroides Jacob On Liver Function In STZ-Induced Diabetic Rats

Table 5 was summarized the Effect Of Coleus Vettiveroides Jacob on liver function in STZ-induced diabetic rats**.** SGPT level was found to be decreased from 82.66±32.00 mg/dl (diabetic control) to 43.77±5.67 mg/dl (Glibenclamide treated groups), 71.67±12.45, and 46.77±18.78 in methanolic of *Coleus Vettiveroides Jacob* extract at the dose of 200 and 400 mg/kg b.wt respectively. The extract was found to be more effective in reducing the SGPT level at the dose of 400 mg/kg b.wt, but less effective when compared to Glibenclamide at the dose of 10 mg/kg b.wt.

ALP level was found to be decreased from 145.18±36.88 mg/dl (diabetic control) to 26.89±15.67 mg/dl (Glibenclamide treated groups), 99.89±28.13, and 38.77±11.67 in methanolic of *Coleus Vettiveroides Jacob* extract at the dose of 200 and 400 mg/kg b.wt respectively. The extract was found to be more effective in reducing the ALP level at the dose of 400 mg/kg b.wt, but less effective when compared to Glibenclamide at the dose of 10 mg/kg b.wt.

Bilirubin level was found to be decreased from 3.11±1.82 mg/dl (diabetic control) to 0.31±0.11 mg/dl (Glibenclamide treated groups), 2.87±1.01, and 1.12±0.78 in methanolic of *Coleus Vettiveroides Jacob* extract at the dose of 200 and 400 mg/kg b.wt respectively. The extract was found to be more effective in reducing the Biliruin level at the dose of 400 mg/kg b.wt, but less effective when compared to Glibenclamide at the dose of 10 mg/kg b.wt.

Effect Of Coleus Vettiveroides Jacob On Kidney Function In STZ-Induced Diabetic Rats

Table 6 shows the effect of coleus Vettiveroides Jacob on kidney function in STZ-induced diabetic rats. Blood Urea level was found to be decreased from 61.22±18.11 mg/dl (diabetic control) to 38.13±9.78 mg/dl (Glibenclamide treated groups), 58.67±21.78, and 41.45±10.88 in methanolic of *Coleus Vettiveroides Jacob* extract at the dose of 200 and 400 mg/kg b.wt respectively. The extract was found to be more effective in reducing the Blood Urea level at the dose of 400 mg/kg b.wt, but less effective when compared to Glibenclamide at the dose of 10 mg/kg b.wt.

Creatinine level was found to be decreased from 9.33±1.12 mg/dl (diabetic control) to 0.65±0.18 mg/dl (Glibenclamide treated groups), 7.89±1.99, and 1.12±0.09 in methanolic of *Coleus Vettiveroides Jacob* extract at the dose of 200 and 400 mg/kg b.wt respectively. The extract was found to be more effective in reducing the Blood Urea level at the dose of 400 mg/kg b.wt, but less effective when compared to Glibenclamide at the dose of 10 mg/kg b.wt.

Effect Of Coleus Vettiveroides Jacob On Pancreatic Function In STZ-Induced Diabetic Rats

Table 7 shows the effect of Coleus Vettiveroides Jacob on pancreatic function in STZ-induced diabetic rats. Serum Amylase was found to be decreased from 110.88±22.07 (diabetic control) to 54.56±10.27 (Glibenclamide treated groups), 98.77±10.89, and 61.89±10.88 in methanolic of *Coleus Vettiveroides Jacob* extract at the dose of 200 and 400 mg/kg b.wt respectively. The extract was found to be more effective in reducing the Serum Amylase level at the dose of 400 mg/kg b.wt, but less effective when compared to Glibenclamide at the dose of 10 mg/kg b.wt.

Liver Glycogen was found to be increased from 8.99±1.45µg/g (diabetic control) to 49.67±6.89, 54.89±11.18µg/g in the groups treated with methanolic of *Coleus Vettiveroides Jacob* at the dose to 200 and 400 mg/kg b.wt respectively. Methanolic of *Coleus Vettiveroides Jacob* at both the doses (200 and 400 mg/kg b.wt) was found to effective in increasing the Liver Glycogen level but when compared to Glibenclamide 88.73±7.67µg/g, it was found to be less effective in restoring the Liver Glycogen level.

Effect Of Coleus Vettiveroides Jacob On Serum (Triglycerides,Total Protein, Total Cholesterol) In STZ-Induced Diabetic Rats

Table 8 represents that the effect of Coleus Vettiveroides Jacob On Serum (Triglycerides,Total Protein, Total Cholesterol) in STZ-induced diabetic rats. Serum triglycerides level were also found to be decreased from 88.34±1.92 mg/dl (diabetic control group) to 67.33±1.79, 71.75±3.78, in diabetic groups treated with methanolic extract at the dose of 200 mg/kg b.wt and 400 mg/kg b.wt respectively. Methanolic extract of *Coleus Vettiveroides Jacob* found to possess good activity in reducing the serum triglyceride level when compared to the diabetic group treated with Glibenclamide (76.45±1.76 mg/dl) at the dose of 10 mg/kg b.wt.

Total protein level was found to be increased from 4.07±0.10 mg/dl (diabetic control) to 7.88±0.56, 7.22±0.25 mg/dl in the groups treated with methanolic of *Coleus Vettiveroides Jacob* at the dose to 200 and 400 mg/kg b.wt respectively. The diabetic group treated with Glibenclamide (10 mg/kg b.wt) almost restored the protein level 8.12±0.45 as that of untreated control groups (8.32±0.36). Methanolic of *Coleus Vettiveroides Jacob* at both the doses (200 and 400 mg/kg b.wt) was found to effective in increasing the total protein level but when compared to Glibenclamide (10 mg/kg b.wt), it was found to be less effective in restoring the total protein level.

Total cholesterol level was found to be decreased from 298.51±1.89 mg/dl (diabetic control) to 120.02±1.10 mg/dl (Glibenclamide treated groups), 136.11±1.79, and 127.54±1.73 in methanolic of *Coleus Vettiveroides Jacob* extract at the dose of 200 and 400 mg/kg b.wt respectively. The extract was found to be more effective in reducing the total cholesterol at the dose of 400 mg/kg b.wt but less effective when compared to Glibenclamide at the dose of 10 mg/kg b.wt.

Table 1: Effect of MECVJ on body weight(gm) in STZ – induced diabetic rats

TREATMENT	*BODY WEIGHT(g)*	
	DAY 1	*DAY 15*
GROUP I	197.02±2.77	223.41±1.73
GROUP II	225.78±3.67	171.34±3.21
GROUP III	215.54±1.86	227.21±1.93
GROUP IV	208.73±3.12	236.75±1.45
GROUP V	218.38±1.98	239.29±1.88

All the values are expressed as mean ±SD (n=5) (P<0.01) significant as compared to standard (diabetic control)

Table 2: Effect of MECVJ on fasting blood glucose level in diabetic rats

Treatment	Fasting blood glucose level (mmol/l)		
	Day 1	Day 7	Day 14
GROUP I	6.52 ±0.06	6.54±0.17	6.71±0.38
GROUP II	22.02±1.07	21.87±2.56	20.87±0.65
GROUP III	23.42±1.23	9.77±0.51***	11.34±0.67***
GROUP IV	23.98±1.12	5.49±0.19***	5.12±0.33***
GROUP V	21.76±1.34	8.32±0.24***	7.39±0.57***

Values are expressed as mean ± SD, n=5, ***p<0.001 when compared to STZ control

Table 3: Effect of MECVJ on OGTT in normal rats

TREATMENT	INITIAL (mg/dl)	30 mins	60 mins	120 mins	240 mins
GROUP I(control) (1 ml distilled water)	73.06±4.03	118.07±8.58	132.61±5.67	140.87±2.19	71.42±2.26
GROUP II(MECVJ (200mg/kg))	71.53±6.41	103.15±10.81	119.67±17.43	112.27±17.09**	81.96±11.02
GROUP III(MECVJ (400mg/kg))	82.62±14.48	113.27±25.15	79.20±12.39**	84.19±10.37**	78.80±13.09**
GROUP IV (GBC 10mg/kg)	70.32±5.98	101.01±8.71	77.45±10.74**	81.23±8.78**	76.55±11.05**

Values are expressed as mean ± SD, *p<0.05 **p<0.01 and ***p<0.001 when compared to normal group

Table 4: Effect of MECVJ on urine glucose level in STZ – induced diabetic rats

Treatment	Intensity of glucose in urine (colour change of the precipitate)				
	DAY 1	DAY 4	DAY 7	DAY 10	DAY 14
GROUP I	Nil	Nil	Nil	Nil	Nil
GROUP II	+++	+++	+++	+++	++++
GROUP III	+++	+++	+++	++	+
GROUP IV	+++	++	++	+	+
GROUP V	+++	++	++	+	+

Keys: (+) = mild, (++) = moderate, (+++) = higher , (++++)=severe,

Table 5: Effect Of Coleus Vettiveroides Jacob On Liver Function In STZ-Induced Diabetic Rats

TREATMENT	SGPT(IU/L)	ALP(IU/L)	BILIRUBIN
GROUP I	39.88±11.12	28.99±7.66	0.22±0.09
GROUP II	82.66±32.00	145.18±36.88	3.11±1.82
GROUP III	71.67±12.45	99.89±28.13**	2.87±1.01
GROUP IV	46.77±18.78**	38.77±11.67**	1.12±0.78*
GROUP V	43.77±5.67	26.89±15.67	0.31±0.11

Values are expressed as mean ± SD,*: Compared with diabetic rats, # : Compared with normal rats, */ # p<0.05, **/ ## p<0.01, ***/ ### p<0.001 when compared to STZ control.

Table 6: Effect Of Coleus Vettiveroides Jacob On Kidney Function In STZ-Induced Diabetic Rats

TREATMENT	BLOOD UREA (mg/dl)	CREATININE (mg/dl)
GROUP I	33.44±5.23	0.87±0.27
GROUP II	61.22±18.11	9.33±1.12
GROUP III	58.67±21.78	7.89±1.99
GROUP IV	41.45±10.88	1.12±0.09**
GROUP V	38.13±9.78	0.65±0.18##

Values are expressed as mean ± SD,*: Compared with diabetic rats, # : Compared with normal rats, */ # p<0.05, **/ ## p<0.01, ***/ ### p<0.001 when compared to STZ control

Table 7: Effect Of Coleus Vettiveroides Jacob On Pancreatic Function In STZ-Induced Diabetic Rats

TREATMENT	Serum Amylase (street-close unit/100ml)	Liver Glycogen (µg/g tissue)
GROUP I	48.77±14.88	72.67±8.99
GROUP II	110.88±22.07	8.99±1.45
GROUP III	98.77±10.89	49.67±6.89**
GROUP IV	61.89±10.88**	54.89±11.18**
GROUP V	54.56±10.27	88.73±7.67##

Values are expressed as mean ± SD,*: Compared with diabetic rats, # : Compared with normal rats, */ # p<0.05, **/ ## p<0.01, ***/ ### p<0.001 when compared to STZ control

Table 8: Effect Of Coleus Vettiveroides Jacob On Serum (Triglycerides,Total Protein, Total Cholesterol) in STZ-Induced Diabetic Rats

Treatment	Triglycerides (mg/dl)	Total Protein (mg/dl)	Total Cholesterol (mg/dl)
GROUP I	56.72±1.32	8.32 ±0.36	123.76±1.43
GROUP II	88.34±1.92#	4.07 ±0.10##	298.51±1.89##
GROUP III	67.33±1.79 ***	7.88 ±0.56 ***	136.11±1.79 ***
GROUP IV	71.75±3.78 ***	7.22 ±0.25 ***	127.54±1.73 ***
GROUP V	76.45±1.76 ***	8.12 ±0.45 ***	120.02±1.10 ***

All the values are expressed as mean ±SD, (n=5) *** P<0.001 as compared to diabetic control, ##P<0.01 as compared to untreated control.

HISTOPATHOLOGICAL STUDIES

Fig 1: Normal control shows pancreas with normal architecture. The islets are composed of normal acini. The acini are lined by round to oval cells with moderate cytoplasm and small round to oval nuclei. There is no evidence of inflammation or necrosis. **Fig 2 :** Diabetic control shows pancreas with engorged and congested blood vessels. The islets show patchy necrosis. The acini are lined by round to oval cells with moderate cytoplasm and small round to oval nuclei. There is mild inflammation composed of lymphocytes. **Fig 3**: Methanolic extract 200 mg/kg of *Coleus Vettiveroides Jacob* shows pancreas. There is a granulomatous infiltrate of lymphocytes within the stroma. The acinar cells are normal. **Fig 4:** Methanolic extract 400 mg/kg *Coleus Vettiveroides Jacob* shows pancreas with normal architecture. The islets are composed of normal acini. The acini are lined by round to oval cells with moderate cytoplasm and small round to oval nuclei. There is an infiltrate of lymphocytes and a few plasma cells within the stroma. **Fig 5:** shows pancreas with normal architecture. The islets are composed of normal acini. The acini are linedby round to oval cells with moderate cytoplasm and small round to oval nuclei.

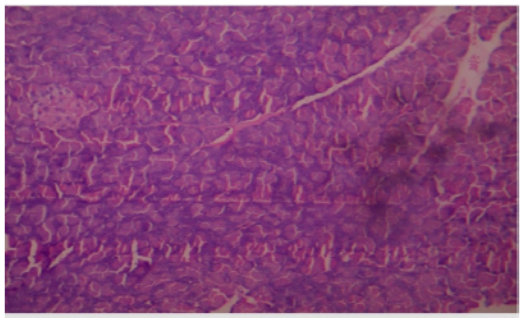

Fig 1: Normal Control

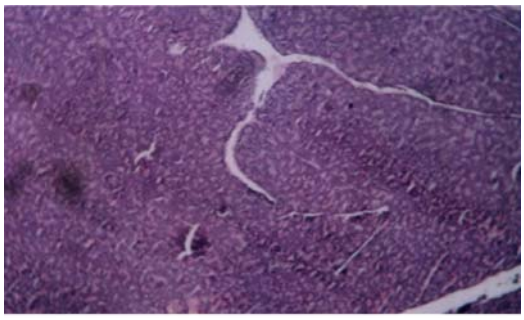

Fig 2: Diabetic Control

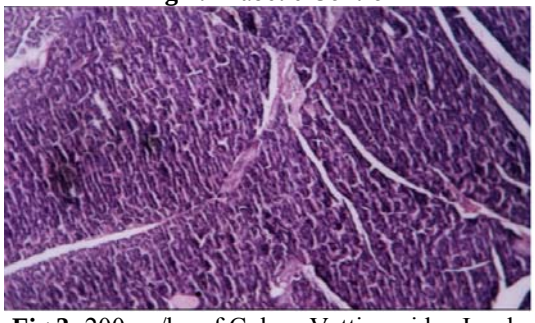

Fig 3: 200mg/kg of Coleus Vettiveroides Jacob

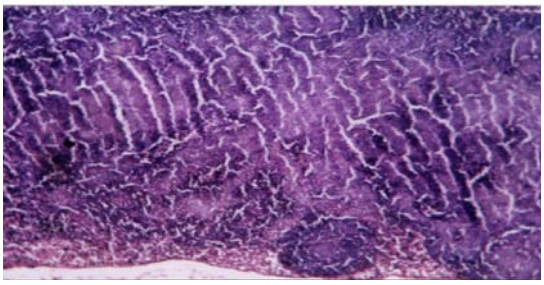

Fig 4: 400mg/kg of Coleus Vettiveroides Jacob

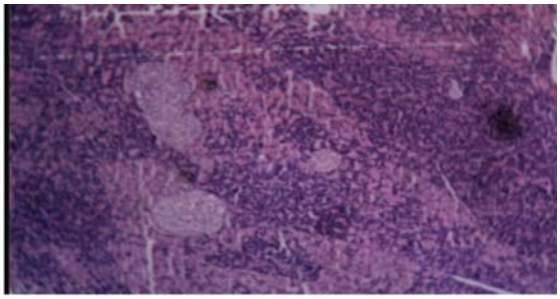

Fig 5: Standard

DISCUSSION

Hyperglycemia 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 casing hyper triglyceridemia. 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 produced a fall in blood glucose level. Hence the methanolic extract of *Coleus Vettiveroides Jacob* 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 sreum cholesterol and triglycerides. The antihyperglycemic activity caused by glibenclamide and MECVJ 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 cholesterogenesis and fatty acid synthesis. Significant lowering of total cholestrol 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[8]. The difference in the body weight observed during the period of treatment of the rats treated with MECVJ 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 gluconeogensis and may also be due to proper glycemic control. Based upon these results it can be hypothesized that MECVJ 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 MECVJ was administered to glucose loaded normal rats fasted for 18 h, reduction in blood glucose levels was observed after 60 minutes. The decline in the level blood glucose reached its maximum at 240 minutes. 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 MECVJ 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[9]. 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[10].The anti-hyperglycemic activity caused by glibenclamide and MECVJ in streptozotocin-induced diabetic rats indicates normalization of serum lipid and stimulation of insulin secretion from beta cells.

Alkaline phosphatase concentration is related to the functioning of hepatocytes, high level of alkaline phosphatase in the blood serum is related to the increased synthesis of it by cells lining bile canaliculi usually in response to cholestasis and increased biliary pressure[15]. Increased levels of bilirubin reflect the depth of jaundice and increased aminotransferases and alkaline phosphatase was the clear manifestation of cellular leakage and loss of functional integrity of the cell[14].The methanolic extract of *Coleus vettiveroides Jacob* and glibenclamide showed a dose dependent activity in reducing the levels of these enzymes. The reversal of increased serum enzymes in streptozotcin-induced liver damage by the extract may be due to its membrane stabilizing activity thus preventing the leakage of intracellular enzymes. The effective control of total protein and total bilirubin can be attributed to an improvement in the hepatic cells' secretory mechanisms.

Several metabolic disorders including urea and creatinine derangements are possible in the presence of streptozotocin over dosage[11].Increased concentration of serum urea and creatinine are considered for investigating drug induced nephrotoxicity in animals and man[12]. Streptozotocin treatment obviously interfered with kidney filtration functions as seen by its elevated values in rats. Urea, a waste product of protein catabolism can rise when the kidney is defective. In renal disease, the serum urea accumulates because the rate of serum urea production exceeds the rate of its clearance[13]. The methanolic extract of *Coleus vettiveroides Jacob* had a dose dependent reversal of the effects on this parameter. Microscopically examined pancreas section of rats treated with methanolic extract showed normal architecture of pancreas. Therefore, on the basis of our results, the possible mechanism of Anti-Diabetic activity of *Coleus Vettiveroides Jacob* might be due to the presence of flavonoids, phenolic compounds, sterols saponins, terpenoids and other active constituents.

CONCLUSION

In the present study, the administration of methanolic extract of *Coleus Vettiveroides Jacob* shows a significant Anti-Diabetic activity in streptozotocin induced diabetic activity on wistar rats. However Further studies are in progress to isolate the active constituents of *Coleus Vettiveroides Jacob* and also to evaluate the exact mechanism of action for the Anti-Diabetic activity.

REFERENCES

1. W. Marshal, S. K. Bangret, Clinical Chemistry Elsevier Limited., 2004, pp. 191-217.

2. Zimmet P, Shaw J, Alberti KGM. Diabetic Medicine, 2003; 20:693-702.

3. Moller DE, Flier J., New England Journal of Medicine, 1991; 325:938-948.

4. R. Vadivelan, M. Dipanjan, P. Umasankar, S. P. Dhanabal, M. N. Satishkumar, S. Antony E. K. Ilango, Advances in Applied Science Research, 2 011 , 2 (3), 179-185.

5. Nash D, Koenig J, Novielli K, Liberoni R, Reisman M. The importance of the individualized pharmaceutical therapy in the treatment of diabetes mellitus. Dis Manag 2001; 4(1): 5-23.

6. Raghunatha Iyer, Indian Medicinal Plants a Compendium of 500 Species. Orient Longman., Delhi. 1994: 4.

7. Harborne J.B. Phytochemical methods. In Chapman &, Hall. New York. 1984; 11: 4-5.

8.Rao Bk, Sudarshan Pr, Rajasekhar MD, Nagaraju N, Rao CA. Anti-diabetic activity of Terminalia pallida fruit in alloxan induced diabetic rat. J Ethnopharmacol 2003;85:169-72.

9. Whtton PD, Hems DA. Glycogen synthesis in perfused liver of streptozotocin diabetic rats. Biochem J 1975;150:153.

10. Maiti, R.,Jana, D., Das, U.K ., Ghosh, D. Anti-diabtic effect of aqueous extract of seed of Tamarindus indica in streptozotocin-induced diabetic rats. J Ethonopharmacol 2004;92:85-1.

11. Kale R H, Halde U K and Biyani K R "Protective Effect of Aqueous Extract of Uraria picta on Acetaminophen Induced Nephrotoxicity in Rats". *Int J Res Pharm Biomed Sci*. 2012; 3 (1): 110-113.

12. Bennit W M, Parker R A, Elliot W C, Gilbert D, Houghton D "Sex related differences in the susceptibility of rat to gentamicin nephrotoxicity". *J. Infec Dis*., 1982; 145: 70-374.

13. Mayne P D "The kidneys and renal calculi In: Clinical chemistry in diagnosis and treatment". London: Edward Arnold Publications. 1994; 6: 2-24.

14.Saroswat B, Visen P K, Patnalik G K and Dhawan B N "Anticholestic effect of picroliv, active hepatoprotective principle of Picrorhizza kurrooa against carbon tetrachloride induced cholestasis". *Ind J. Exp Biol*.,1993; 31: 316- 318.

15. Handa SS, Sharma A. Hepatoprotective activity of andrographolide from Andrographis paniculata against CCl4. *Ind. J. Med Res* (B) 1990; 92: 276-83.

16. Felter HW, MD, Lloyd JU. Bryonia (U.S.P). King's American Dispensatory 1898.

17. Lowry OH, Farr A.L and Randall R.J. Protein measurement with folin phenol reagent. *J.Biol.Chem*. 1951; 193: 265-275.

18.Hassid,W.Z., Abraham S. Chemical Procedures For Analysis Of Polysaccharide. Colowick, Kapalan S.P. Method In Enzymology,New York, Academic Press; 1975: (3)34-36.

19. Kind P.R and King R.J. Estimation of plasma phosphatase by determination of hydrolysed phenol with antipyrin*. J.clin.path*.1954; 7: 322-326.

20. Reitman S and Frankel S. A Colorimetric method for determination of serum glutamate oxaloacetate and glutamic pyruvate transaminase activity. Am. *J. Clin. Path*. 28; 1957: 56-58.

21. Malloy and Evelyn. The determination of bilirubin with the photoelectric colorimeter., J.Bio.Chem., 1937;(119): 481-490.

22. Street, H.V. and Close, J.R. (1956), clin.chem.Acta.,1,256. Textbook of practical Physiology, Chaudhari (2000).

23. Toro,G. and Ackermann., P.G. (1975) , practical clinical chemistry, Little brown &co., Boston , p154.