Available online at www.ijpsdr.com
International Journal of Pharmaceutical Sciences and Drug Research 2013; 5(2): 50-55

Research Article ISSN 0975-248X

Study of Hypoglycemic Activity of Aqueous Extract of *Leucas indica* Linn. Aerial Parts on Streptozotocin Induced Diabetic Rats

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ABSTRACT
The present study was designed to evaluate the hypoglycemic activity of the aqueous extract of *Leucas indica* Linn. on streptozotocin induced diabetic rats. The extract showed a significant dose depended (200 and 400 mg/kg b.w, orally) reduction in fasting blood glucose level, comparing with reference drug, glibenclamide (0.5 mg/kg b.w, orally). In addition, the changes in body weight, analysis of serum biochemical parameters like lipid profile, glutamate oxaloacetate transaminase, glutamate pyruvate transaminase, total proteins, bilirubin, urea and alkaline phosphatase were assessed for each group of animals and the significant differences were observed when compared with control groups. Finally, in case of histopathological investigation of pancreas, liver and kidney from each group of rats showed a significant protection and regeneration of earlier streptozotocin induced cellular necrosis. This finding proves that the extract exhibits sufficient hypoglycemic activity along with improves streptozotocin induced negative body weight as well as normalize serum biochemical parameters and protect from cellular necrosis significantly.

Keywords: *Leucas indica*, Aqueous Extract, Hypoglycemic, Streptozotocin, Biochemical Parameters, Histopathology.

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia, glycosuria, hyperlipemia, negative nitrogen balance and sometimes ketonemia, resulting from an absolute deficiency of serum insulin level due autoimmune antibody induced destruction of insulin secreting β-cells of pancreatic islets of langerhans (IDDM) or resulting from resistance to insulin release from β-cells as well as desensitization of peripheral tissue to insulin and down regulation of insulin receptors (NIDDM). [1] It is well known fact that, the incidence of diabetes mellitus is very high throughout the world and it is more common in South-East Asia. Presently, the most common anti-diabetic drugs like sulfonylurea, thiazolidinedione and biguanides produce several adverse effects. [2] Therefore, several new approaches are now considered for the development of superior anti-diabetic agents from herbal sources to avoid the adverse effects of current synthetic medication. [3] Even the World Health Organization (WHO) suggests the use of herbal medication for diabetes mellitus as it is much safer in concern. [4] In case of experimental induced diabetes mellitus, Streptozotocin (STZ) is widely used as it produces reactive oxygen species (ROS) resulting, toxicity, oxidative stress and necrotic events on β-cells of pancreatic islets of langerhans. [5]

The plant *Leucas indica* Linn., belonging to the family, Labiatae is commonly known as 'Dandokalos' in Bengali, is distributed in throughout the India, in the road sides, waste lands, river banks, on rocky hills and abundantly present in 'Mahananda Neora Valley' in West Bengal. The herbs are almost erect, pubescent branching, leaves are linear-lanceolate, flowers are white, calyx tube slightly curved, corolla is annulated and stamens are four. [6] Traditionally, the leaves of this plant are used as vermifuge, stomachic, sedative and in sores. [7] The aerial parts of *Leucas indica* Linn. contain phenylethanoid glycosides, having antioxidant, Xanthene oxidsase inhibition as well as wound healing activity. [8-9] However, based on literature survey and traditional use, the present study was designed to evaluate the hypoglycemic activity of aqueous extract of *Leucas indica* Linn. on STZ induced diabetic rats.

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MATERIALS AND METHODS

Plant Material

The aerial parts of *Leucas indica* Linn. were collected in August, 2011 from Duars region, Jalpaiguri District, West Bengal, subsequently identified and authenticated from Central National Herbarium, Botanical Survey of India,

Howrah-711103, West Bengal (Ref No. CNH/32/2012/Tech.II/625 Dated: 06.03.2012). After proper washing, it was dried under shade at a room temperature for seven days and then grinded with a mechanical grinder. Finally, the coarse powders were separated by sieving using 40 mesh and stored in an air tight container for further use.

Preparation of Plant Extract

The fresh coarse powders were subjected to maceration by petroleum ether to remove fatty materials and then successively extracted with chloroform, methanol and distilled water according to ascending order of polarity of solvent using a Soxhlet apparatus. The each fraction of the extract was then filtered and concentrated to dryness in a rotary vacuum evaporator under reduced pressure and temperature and stored in desiccators. During performing the experiment, the dried aqueous extract was dissolved in distilled water to prepare the subsequent useable extract (LIAE). The preliminary phytochemical screening of LIAE done by the method mention by Harbone and Trease confirmed the presence of flavonoids, total phenolic compounds, saponin and tannin. [10-11]

Chemicals and Reagents

Streptozotocin was purchased from SRL Pvt. Ltd. (Mumbai), glibenclamide from Sigma Chemicals (Mumbai), the reagent kit for the measurement of serum glutamate oxaloacetate transaminase (SGOT), glutamate pyruvate transaminase (SGPT), triglyceride, cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), creatinine, total protein, urea, bilirubin, alkaline phosphatase from SPAN Diagnostic Pvt. Ltd (Mumbai). The remaining solvents and chemicals were used for this study are in analytical grade.

Animals

Wistar albino rats (weighing 150-200 g) of either sex were used to perform this experiment. The animals were randomly grouped (n=6) and housed in polyacrylic cages (38×23×10 cm) and maintained under standard laboratory conditions (Temp. 25 ± 2ºC) with dark and light cycle (14/10 h). They were allowed freely to access the standard diet in the form of dry pellet (purchased from Hindustan Lever, Pvt. Ltd. Kolkata) and water *ad libitum*. The rats were acclimatized to standard laboratory condition for 1 week before commencement of this experiment. The ethical clearance was obtained from the 'Jadavpur University Animal Ethical Committee' for using animals in the present study (Vide No. 0367/01/C/CPCSEA, India).

Toxicity study

Wistar albino rats (weighing 150-200 g) of either sex were divided into several groups containing 10 animals of each. Different doses of LIAE (200, 500, 1000, 1500, 2000, 2500, 3000, 3500 mg/kg b.w) were administered orally to the treated groups but control groups received only normal saline orally (5 ml/kg b.w) under overnight fasting condition. The sign of toxicity and mortality were recorded within 24-72 h for all groups of animals. The LD_{50} was determined using graphical representation and probit analysis. [12]

Oral Glucose Tolerance Test

The oral glucose tolerance test (OGTT) was performed in normal rats after overnight fasting condition (18 h) with free access of water. The animals were divided into four groups of six animals in each. The group-I was marked as normal control, administered with normal saline orally (0.9% w/v NaCl, 5 ml/kg). The group-II and III were treated with LIAE orally at the dose level of 200 and 400 mg/kg b.w respectively and group-IV received the reference drug, glibenclamide (0.5 mg/kg b.w, orally). After 30 min of this treatment, a single dose of glucose solution (2 gm/kg b.w) was administered orally to the all animals including control group to increase instant blood glucose level. The blood glucose level was measured at 0 min (just prior to glucose administration) 30, 60, 90, 120 and 240 min (after glucose feeding) by digital glucometer (Accu-Chek Active, India model) on the basis of glucose oxidase mechanism. [13] The blood samples were collected from tip of the tail vein by sharp incision under mild anaesthetic condition.

Induction of Diabetes

All the animals excluding normal control group were injected intraperitoneally by the fresh solution of STZ (40 mg/kg b.w) in 0.1 M cold citrate buffer, pH 4.5 after overnight fasting condition whereas the normal control animals were injected with citrate buffer alone (5 ml /kg b.w) in same route. [14] About 5% w/v of oral glucose solution was administered on first day after STZ administration to prevent hypoglycemic shock. Diabetes state was confirmed by the elevation of fasting blood glucose level after 48 hr of following STZ administration. The animals with a fasting blood glucose levels in the range of 350–425 mg/dl were selected for this study.

Hypoglycemic activity by acute model

The protocol used here for acute hypoglycemic activity study of Datusalia method with little modification. [15] The overnight fasting animals were divided into five groups of six animals in each. Group-I (normal control) and Group-II (diabetic control) received a single dose of normal saline (5 ml/kg b.w, orally). Group-III and IV received LIAE, orally at a single dose of 200 and 400 mg/kg b.w respectively. Group-V received glibenclamide at a single dose of 0.5 mg/kg b.w, orally. The fasting blood samples were collected from tip of the tail vein under mild anaesthetic condition at 0, 1, 3, 5, 7 and 9 hr after the oral administration of respective dose and the glucose levels were determined by digital glucometer.

Hypoglycemic activity by Sub-acute model

The sub-acute model was almost similar to acute model but instead of 9 h observation, the treatment was continued once daily for 15 days period. [16] The fasting blood glucose level was recorded on day 1, 4, 7, 10 and 15 after initiation of experiment. The body weights of each group of animals were also recorded on day 1, 4, 7, 10, 13 and 15.

Measurement of biochemical parameters

On the day of 15^{th}, the blood samples were collected from the overnight fasting animals by retro-orbital bleeding using micro-capillary technique under mild anesthetic condition. [17] After centrifugation, the serum was separated for spectroscopic analysis by using automated diagnostic reagent kit to determine the serum biochemical parameters, such as glutamate oxaloacetate transaminase (SGOT), glutamate pyruvate transaminase (SGPT), triglycerides, total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), creatinine, total proteins, urea, total bilirubin, alkaline phosphatase. [18]

Histopathological Study

Finally, on the day of 15^{th} the animals of each group were sacrificed to dissect out the tissues of pancreas, liver and kidney and preserved in formalin solution immediately. The harvested tissues were then subjected to very thin section by using microtome instrument and fixed in 10% neutral formalin solution, embedded in paraffin, stained with

Table 1: Effect of LIAE on oral glucose tolerance test on rats

Group	Blood glucose level (mg/dl) at different time intervals					
	0 min	30 min	60 min	90 min	120 min	240 min
Gr-I Normal Control	87.00 ± 1.47	151.50 ± 3.44	130.00 ± 1.69	117.20 ± 3.36	111.30 ± 2.78	91.50 ± 2.77
Gr-II LIAE 200	85.83 ± 2.21	138.00 ± 3.96***	117.70 ± 2.17***	107.00 ± 2.49*	95.83 ± 1.35	88.00 ± 3.30
Gr-III LIAE 400	87.67 ± 2.73	130.30 ± 2.51***	116.80 ± 2.58***	102.30 ± 1.86*	87.67 ± 1.50	77.67 ± 1.61
Gr-IV Glibenclamide	88.67 ± 2.19	131.30 ± 3.77***	107.70 ± 2.86***	99.67 ± 1.52*	84.00 ± 1.34	74.33 ± 2.54

Values are expressed as Mean ± SEM; n= 6 ; *P< 0.05; ***P< 0.001; LIAE: Aqueous Extract of *Leucas indica*

Table 2: Effect of LIAE on blood glucose level in acute model

Group	Blood glucose level (mg/dl) at different time intervals					
	0 h	1 h	3 h	5 h	7 h	9 h
Gr-I Normal Control	87.67 ± 3.31	89.5 ± 3.23	82 ± 2.11	84.5 ± 4.05	85 ± 2.23	85.83 ± 2.54
Gr-II Diabetic control	392.30 ± 9.23	388.30 ± 8.54**	393.70 ± 7.00**	386.20 ± 7.16**	383.30 ± 3.60**	391.30 ± 7.91**
Gr-III LIAE 200	387.20 ± 10.77	338.50 ± 9.19*	327.00 ± 9.47*	295.50 ± 10.06*	278.50 ± 9.45*	253.80 ± 13.45*
Gr-IV LIAE 400	395.50 ± 8.79	366.00 ± 10.69*	335.20 ± 7.96*	304.20 ± 4.534*	263.30 ± 4.21*	236.20 ± 5.90*
Gr-V Glibenclamide	391.30 ± 14.87	359.50 ± 15.54*	339.00 ± 15.78*	322.80 ± 15.69*	296.80 ± 17.86*	272.20 ± 21.64*

Values are expressed as Mean ± SEM; n= 6; *P< 0.05; **P< 0.01; LIAE: Aqueous Extract of *Leucas indica*

Table 3: Effect of LIAE on fasting blood glucose level in sub-acute model

Group	Fasting blood glucose level (mg/dl) at different day intervals					
	1st day	4th day	7th day	10th day	13th day	15th day
Gr-I Normal Control	83.33 ± 2.16	92.17 ± 2.21	83.00 ± 2.68	86.17 ± 3.31	85.17 ± 1.89	83.00 ± 2.19
Gr-II Diabetic control	374.20 ± 6.65	390.30 ± 5.82***	397.70 ± 6.08***	401.70 ± 12.07***	428.30 ± 9.98***	448.70 ± 11.9***
Gr-III LIAE 200	342.70 ± 16.67	304.80 ± 12.13**	263.30 ± 11.03**	213.00 ± 10.43**	163.00 ± 12.35**	96.67 ± 4.72**
Gr-IV LIAE 400	326.00 ± 17.41	276.70 ± 18.59**	233.20 ± 18.41**	182.70 ± 17.41**	136.00 ± 15.88**	87.17 ± 3.351**
Gr-V Glibenclamide	345.50 ± 18.03	298.00 ± 17.19**	248.80 ± 17.91**	198.20 ± 17.24**	147.80 ± 14.95**	90.00 ± 5.89**

Values are expressed as Mean ± SEM; n=6; **P< 0.01; LIAE: Aqueous Extract of *Leucas indica*

Table 4: Effect of LIAE on body weight

Group	Body weight in g at different day intervals					
	1st day	4th day	7th day	10th day	13th day	15th day
Gr-I Normal Control	160.0 ± 7.23	164.4 ± 6.23	168.6 ± 4.69	171.0 ± 5.18	173.4 ± 5.24	178.0 ± 4.95
Gr-II Diabetic control	163.3 ± 5.11**	159.5 ± 5.14**	155.3 ± 4.57**	149.2 ± 4.67**	145.3 ± 4.53**	140.3 ± 4.26**
Gr-III LIAE 200	158.2 ± 6.95**	154.3 ± 6.64**	153.3 ± 6.65**	153.8 ± 6.16**	155.8 ± 6.54**	155.8 ± 5.34**
Gr-IV LIAE 400	153.7 ± 6.23**	152.5 ± 5.57**	152.0 ± 5.80**	156.2 ± 5.77**	156.5 ± 5.93**	161.3 ± 6.28**
Gr-V Glibenclamide	157.3 ± 5.84**	156.7 ± 5.16**	157.5 ± 5.80**	157.8 ± 7.64**	156.3 ± 7.32**	156.5 ± 6.14**

Values are expressed as Mean ± SEM; n= 6; **P< 0.01; LIAE: Aqueous Extract of *Leucas indica*

Table 5: Effect of LIAE on serum biochemical parameters

Biochemical Parameters	Gr-I Normal Control	Gr-II Diabetic control	Gr-III LIAE 200	Gr-IV LIAE 400	Gr-V Glibenclamide
SGOT (IU/L)	30.33 ± 0.58	97.73 ± 1.22**	57.00 ± 1.56**	48.28 ± 1.10**	39.33 ± 2.38**
SGPT (IU/L)	12.88 ± 0.44	75.10 ± 0.30**	42.76 ± 0.39**	36.32 ± 0.47**	25.69 ± 0.41**
TG (mg/dl)	101.50 ± 1.35	272.70 ± 4.56**	182.80 ± 0.92**	175.00 ± 0.60**	147.50 ± 1.53**
TC (mg/dl)	172.70 ± 1.48	306.70 ± 2.10**	225.80 ± 1.79**	197.00 ± 2.02**	184.90 ± 1.47**
HDL (mg/dl)	111.90 ± 0.84	34.71 ± 0.46**	52.87 ± 1.08**	75.41 ± 0.89**	86.26 ± 0.94**
LDL (mg/dl)	40.44 ± 1.22	218.70 ± 0.96**	137.80 ± 1.69**	84.66 ± 0.29**	67.01 ± 0.87**
SCr (mg/dl)	0.78 ± 0.04	1.53 ± 0.19**	1.47 ± 0.22**	0.96± 0.06**	0.84± 0.08**
TSP (g/dl)	7.43 ± 0.26	4.86 ± 0.23**	6.54 ± 0.23**	7.64 ± 0.27**	9.50 ± 0.29**
SU (mg/dl)	15.67 ± 1.41	63.17 ± 2.15**	44.83 ± 0.83**	34.83 ± 2.23**	18.83 ± 1.25**
TBl (mg/dl)	0.23 ± 0.02	1.58 ± 0.16**	0.84 ± 0.03**	0.55 ± 0.02**	0.44 ± 0.02**
SALP (KA units/dl)	6.32 ± 0.33	23.1 ± 0.68**	11.94 ± 0.57**	9.81 ± 0.59**	7.71 ± 0.25**

Values are expressed as Mean ± SEM; n= 6; **P< 0.01; LIAE: Aqueous Extract of *Leucas indica*; GLI: Glibenclamide; SGOT: serum glutamate oxaloacetate transaminase; SGPT: serum glutamate pyruvate transaminase; TG: serum triglycerides; TC: serum total cholesterol; HDL: high density lipoprotein; LDL: low density lipoprotein; SCr: serum creatinine; TSP: total serum proteins; SU: serum urea; TBl: serum total bilirubin; SALP: serum alkaline phosphatase.

hematoxylin (H) and eosin (E) and finally photographs were taken by using binocular microscope. [19]

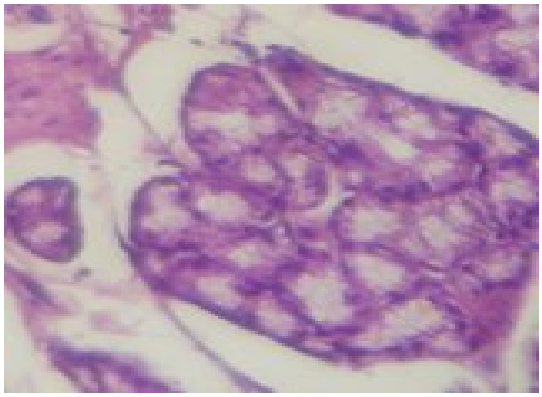
Statistical analysis

The results were expressed as mean ± SEM. Statistical differences between the treated and control groups were determined by one way ANOVA followed by Dunnet's test using the computer software, Graph Pad Prism 5 verson. The P values less than 0.05 were considered as statistically significant.

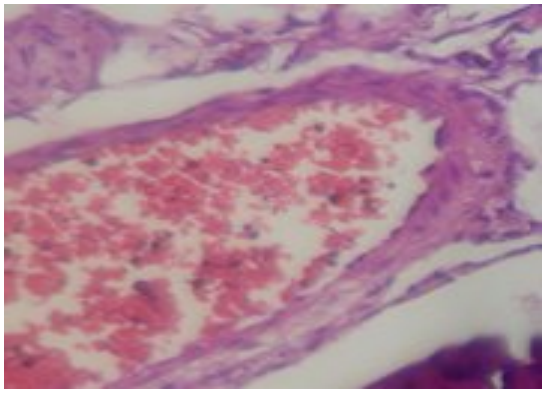
RESULTS AND DISCUSSION

In acute toxicity study the LD_{50} values was 2630 mg/kg b.w (by oral route) for LIAE. However no significant visual toxicity was found up to the dose of 1500 mg/kg b.w for the extract. The oral glucose tolerance test (OGTT) was performed in normoglycemic rats where the LIAE and glibenclamide showed significant ($P < .05$) dose dependent reduction of the progressively elevated blood glucose level in glucose loaded hyperglycemic rats (Table 1). It is already reported that the reference drug glibenclamide reduces blood glucose level by stimulating pancreatic β-cells to release more insulin. The hypoglycemic activity of LIAE may be due to reduction of the intestinal absorption of glucose or may be able to increase the utilization of glucose by the peripheral tissues or by similar fashion as glibenclamide.

In case of both acute and sub-acute model, the LIAE produced a significant (P< 0.05 and P< 0.01) dose dependent reduction in fasting blood glucose level when compared to the control groups (Table 2 and 3). Usually the normal control rats gained their body weight during 15 days period, but all others group showed decrease in body weight over the same period of observation.



(1a)



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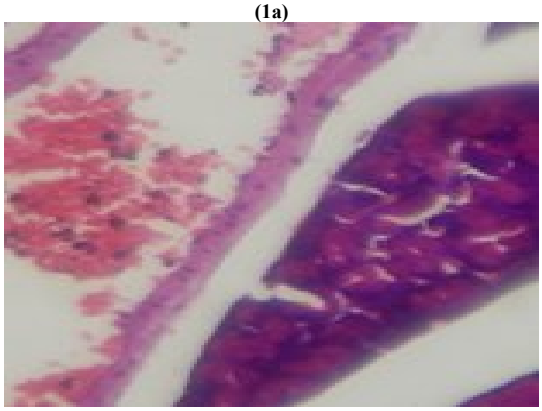

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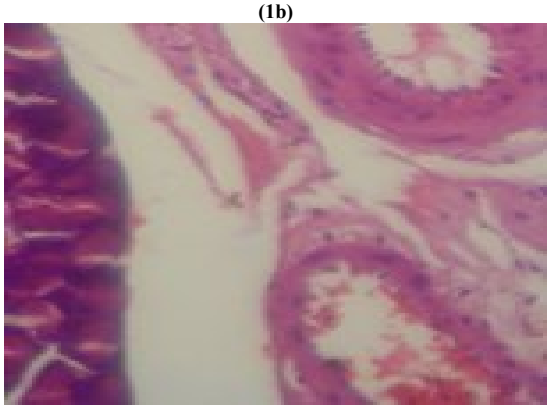
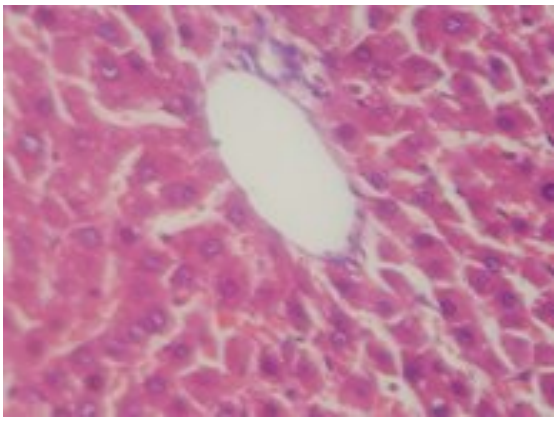
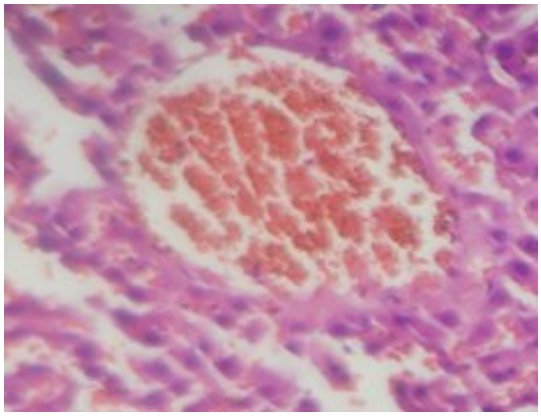


(1d)

Fig. 1a: Normal control pancreas; Fig. 1b: Diabetic control pancreas; Fig. 1c: LIAE treated pancreas; Fig. 1d: Glibenclamide treated pancreas Microscopical Photograph (H and E x 10)



(2a)



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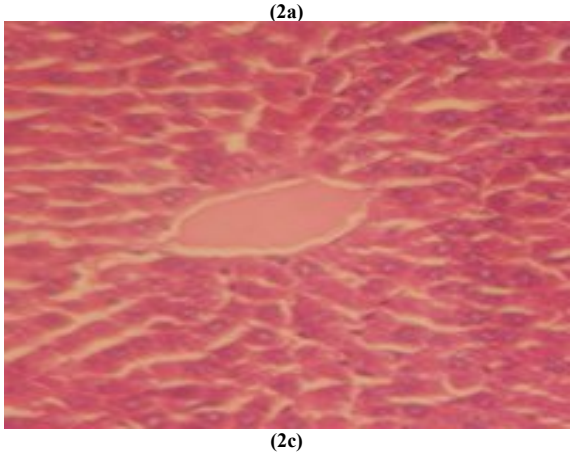

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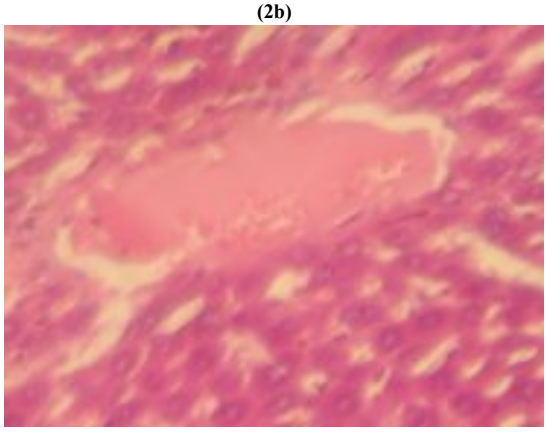
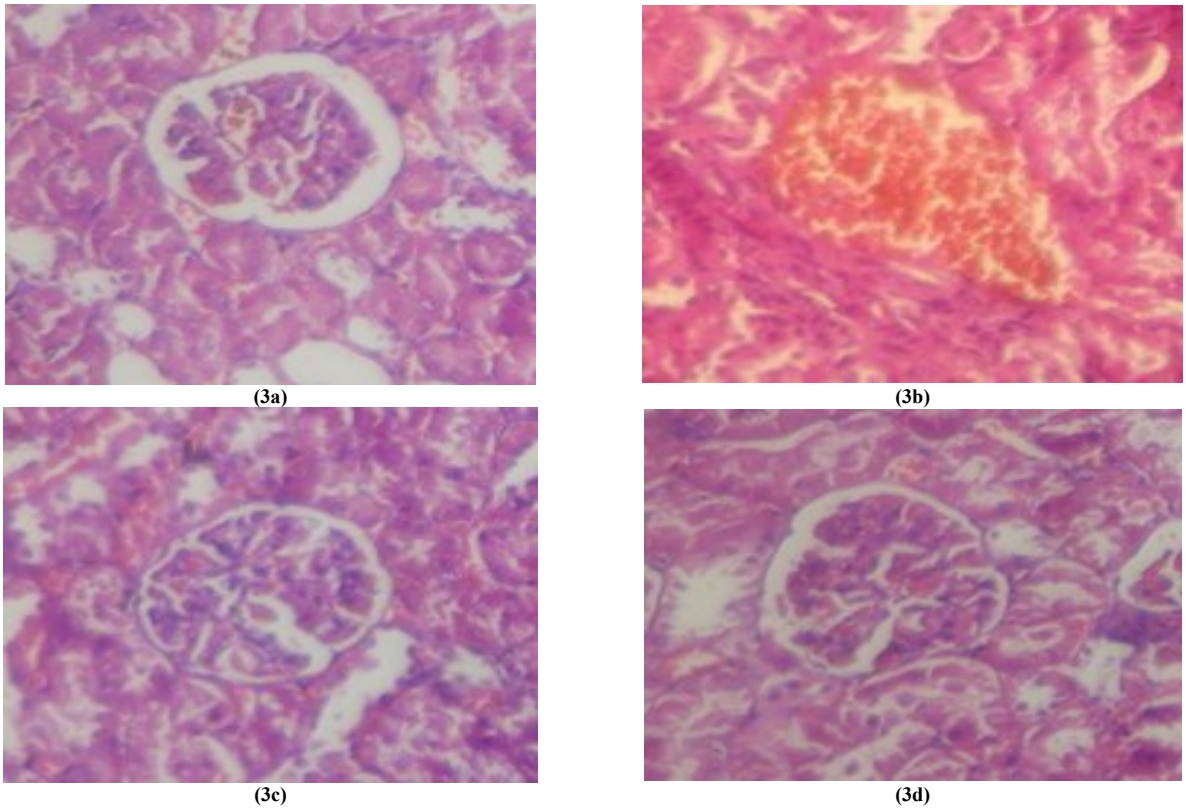


(2d)

Fig. 2a: Normal control Liver; Fig. 2b: Diabetic control Liver; Fig. 2c: LIAE treated Liver; Fig. 2d: Glibenclamide treated Liver Microscopical Photograph (H and E x 10)



(3a) **(3b)**

(3c) **(3d)**

Fig. 3a: Normal control Kidney; Fig. 3b: Diabetic control Kidney; Fig. 3c: LIAE treated Kidney; Fig. 3d: Glibenclamide treated Kidney Microscopical Photograph (H and E x 10)

The both LIAE and glibenclamide treated diabetic animals gained significant ($P< 0.01$) body weight but lesser than normal control rats (Table 4). The diabetic induced agent, STZ destroys the pancreatic β-cells by selective degeneration and necrosis as well as impairs glucose utilization by the peripheral tissue causing hyperglycemia in animals. [20] The possible mechanism of LIAE on hypoglycemic effect in acute and sub-acute models may be similar to glibenclamide or due to increased peripheral utilization of glucose or due to increased glycogenesis and decreased both glycogenolysis as well as endogenous glucose production in liver. [21] The body weight generally decreases in diabetic state, but LIAE significantly increased body weight probably by inhibition of gluconeogenesis and glycogenolysis. [22] In case of observed biochemical parameters the glutamate oxaloacetate transaminase (SGOT), glutamate pyruvate transaminase (SGPT), alkaline phosphatase, creatinine, urea, bilirubin, low density lipoprotein (LDL), triglycerides and cholesterol levels were elevated in diabetic control animals but showed significant dose dependent reduction ($P< 0.01$) both in LIAE and reference group due to protective action of STZ induced organ damage that shows elevation of serum oxaloacetate transaminase, glutamate pyruvate transaminase, alkaline phosphatase and creatinine. On the other hand, the level of serum high density lipoprotein (HDL) and total proteins were lowered in diabetic control animals where as LIAE and reference groups showed higher serum high density lipoprotein and total proteins level (Table 5). In case of diabetes mellitus there is impaired synthesis of glycogen by liver resulting elevation of serum low density lipoprotein, triglycerides and cholesterol level. [23] The LIAE may reactivate the glycogen synthetase system or may inhibit glycogenolysis and gluconeogenesis in liver as well may cause conversion of low density lipoprotein to high density lipoprotein. Finally in case of histopathological study the diabetic control animals showed degenerative changes and necrotic events in pancreas, liver and kidney tissues but in case of LIAE and glibenclamide treated animals showed maximum cellular regeneration and increased numbers of β-cells, hepatic cells and glomerular cell respectively (Fig: 1-3). It is already reported that the flavonoids show antioxidant activity by directly scavenging the free radicals and thus can prevent oxidative stress induced damage of vital organs like pancreas, liver and kidney. [24] In the present study, the LIAE showed the protective action against oxidative stress induced damage of these organs. Therefore, it could be concluded that, probably the flavonoids present in LIAE having a major role in reducing oxidative stress that causes degenerative change and necrosis in diabetic state.

ACKNOWLEDGEMENT

The authors are thankful to the Scientist, Botanical Survey of India, Central National Herbarium, Botanical Garden, Howrah-711103, West Bengal for identification and authentication of the plant.

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