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Evaluation of protective effect of *Centella asiatica* leaves on pancreas function in diabetic rats

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Abstract
Centella asiatica is a well-known plant with a wide range of medicinal properties. The aim of the study is to investigate the protective effect of *Centella asiatica* leaves on pancreas function in experimental diabeticrats. Diabetes was induced in rats by injecting streptozotocin at a dose of 50 mg/kg body weight (b.w) intraperitoneally. The experimental rats were treated with the methanol extract of *Centella asiatica* leaves (CALEt) at a dose of 300mg/kg b. w. After the treatment period all the animals were sacrificed, and the blood sample was analyzed for insulin, glucagon, glycosylated haemoglobin and other blood parameters. Oral administration of CALEt for a period of 3o days restored the altered parameters. Histology of diabetic rats treated with CALEt showed pancreatic β-cell regeneration. Thus, our results revealed that the administration of CALEt may have protective effect on the pancreas of STZ induced diabetic rats.

Keywords: Diabetes mellitus, *Centella asiatica*, pancreas, insulin, glucagon

1. Introduction
Diabetes mellitus isa metabolic disorder and it is a major threat to the healthcare universally. WHO estimates that in 2012, roughly 1.5 million deaths were absolutely caused by diabetes and 80 percent of these deaths occurred in low and middle-income countries [1]. Diabetes is a chronic disease that arises when the pancreas does not secrete enough insulin, or when the body cannot effectively make use of the insulin, it produces. Hyperglycemia is a common effect of uncontrolled diabetes and ultimately leads to serious damage to various body's systems. The pancreas plays a major role in the metabolism of glucose by secreting the hormones insulin and glucagon. Both are polypeptide hormones secreted in the pancreatic islets of Langerhans. However, insulin is produced by the β cells in response to a rise in blood-glucose and glucagon is produced by α cells and it is secreted in response to low blood-glucose level (hypoglycemia). Insulin is necessary for proper regulation of glucose and for maintenance of perfect blood-glucose levels [2]. Generally, glucagon opposes the action of insulin. It increases blood-glucose concentration partly by breaking down the stored glycogen in the liver by a pathway known as glycogenolysis and gluconeogenesis. At present, several approaches are available in the market for the treatment of diabetes such as sulfonylureas, biguanides, α-glucosidase inhibitors, insulin, etc. All the existing treatments are often associated with undesirable side effects and did not give complete or permanent cure for the disease. Currently, focus on plant research has increased throughout the world and considerable proof has been accumulated to highlight the massive potential of medicinal plants, used in various traditional systems of medicine [3, 4]. *Centella asiatica* (L.) Urbanis a slender, stonoliferous, creeping perennial herb belonging to the Apiaceae family (Umbelliferae) and sub-family Hydrocotyle, growing in the soggy areas in different tropical countries. It has been reported to have a wide range of biological activities preferred for human health such as anti-ulcer [5], wound healing [6–8] anti-inflammatory [9, 10], anti-viral [11], Hepatoprotective [12], antipsoriatic [13], cytotoxic and anti-tumor [14, 15], sedative [16], immunostimulant [17], anticonvulsant [18], cardio protective [19, 20], insecticidal [21], antidiabetic [22], antibacterial [23], lepra [24], antifungal [25] and venous deficiency treatments [26, 27]. There are several claimson the subject of theprimarymechanism involved in the biological action of this herb, more scientific data is required to justify its ever-increasing use. Therapeutic potential of this plant in terms of its effectiveness and adaptability is such that more detailed investigation would appear crucial. Present study was undertaken to investigate the effect of, glucagon, total haemoglobin, glycosylated haemoglobin, RBC count and WBC countins methanol extract of *Centellaa sciatica* leaves on body weight, blood glucose, in sulintreptozotocin induced diabetic rats by comparing the results with standard anti-diabetic drug, glibenclamide. In addition, histology of pancreas was studied in both normal and experimental rats.

2. Materials and Methods

2.1 Plant material

The whole plant of *Centella asiatica* (L.) was collected from Talakona forest in the Chittoor district of Andhra Pradesh, India during November 2009. The plant was authenticated by ataxonomistin the Department of Botany, S.V University, and a voucher specimen (No: 1976) was deposited in the S.V. University herbarium, Tirupati, Andhra Pradesh, India.

2.2 Animals

Male Wistar albino rats, weighing (160 ± 20 g) were purchased from the authorized dealer (Venkateswara enterprises, Bangalore). The experimental protocol was approved by the Institutional Animal Ethical Committee at Sri Venkateswara University, Tirupati, Andhra Pradesh, India (R.No.10/I/A/CPCSEA/IACE/SVU/PSR-MRA/10.06.2010). The rats were maintained in ventilated clean polypropylene cages. The rats were provided with a commercial standard pellet diet ad libitum and water. The experiments were carried out in harmony with the CPCSEA (Committee for Control and Supervision on Experiments on Animals) guidelines, Government of India.

2.3 Chemicals

All chemicals used in the current study were Analytical Grade (AR) and procured from the following companies: Sigma (St. Louis, MO, USA), Sanofi–Aventis (USA), Merck (Mumbai, India), BDH Ltd (England) and Ranbaxy (New Delhi, India).

2.4 Plant extract preparation

Fresh leaves of *Centella asiatica* were washed with distilled water and shade dried for four weeks. After drying the plant material was minced into fine powder by means of a mixer grinder. The powdered material (2 kg) was drenched in 6 L methanol for 48 hrs and filtered. The extracted material was filtered through what man number 1 filter paper. The obtained filtrate was collected and evaporated in a rotary evaporator at 40-50 °C under reduced pressure. The material was dried in vaccum desicator and stored in refrigerator at 4°C until use. (Yield: 9.86 % w/w)

2.5 Induction of experimental diabetes mellitus

Animals were fasted for overnight, 12 hours before injection. They were induced by a single intraperitoneal injection of streptozotocin 50 mg/kg b. w [28] dissolved in 0.1 M cold citrate buffer, (P^H 4.5). The animals were allowed to drink 15% glucose solution for 72 hrs to overcome the drug induced hypoglycemia. After injection, animals had free access to food and water. The rats with blood glucose values above 250 mg/dl were considered as diabetic on day 3after STZ injection. Subsequent to diabetes confirmation, rats were allowed for seven days to acclimatize to diabetic condition, and rats with moderate diabetes (blood glucose > 250 mg/dl) were considered to be diabetic and used for the research. Treatment was started on eighth day after STZ injection which was also considered as the initial day of treatment and continued further until end of the study period (30 days).

2.6 Experimental Design

Rats of the same age group (3 months old) were divided into 5 groups, six rats in each group

Group I: Normal control rats received distilled water for equivalent handling.

Group II: Normal rats received (methanol extract of *Centella asiatica* leaves) CALEt daily for a period of 30 days at a dose of 300 mg/kg b. w. [29]

Group III: Diabetic untreated rats which served as diabetic controls. This group of rats received distilled water for equivalent handling.

Group IV: Diabetic rats treated with CALEt for a period of 30 days at a dose of 300 mg/ kg b. w.

Group V: Diabetic rats treated with the standard diabetic drug, glibenclamide at a dose of 5 mg/kg b.w [30], for the same period as in group IV.

Blood glucose levels were recorded weekly during the experiential period. The body weight of all the rats was measured at days 0, 15 and 30.

2.7 Biochemical estimations

Estimation of blood glucose was carried out by using dextrose strips (Glucose oxidase – peroxide method) with one touch glucometer (Manufacture: Johnson and Johnson).After completion of 30 days of treatment, the animals were sacrificed by cervical dislocation and blood was collected by cardiac puncture. A part of blood was collected into EDTA bottles for haematological determinations *viz*, total haemoglobin, glycosylated haemoglobin, RBC Count and WBC Count. The estimation of hemoglobin was carried out by the method of Drabkin and Austin [31].RBC was counted by manual procedure using the hemocyto meter Neubauer chamber and White blood cells are counted using the manual procedure, using a diluting chamber and a hemocyto meter. Another portion of blood sample was put into test tubes and allowed to clot at room temperature for 1 hr then put in a refrigerator for further one hour. The serum was collected after centrifugation at 2000 x g for 15 min. The serum aspirated off for biochemical evaluation *viz*, insulin and glucagon. Glycosylated haemoglobin Insulin and glucagon were estimated by using ELISA (enzyme linked immune-sorbent assay) kit.

2.8 Histopathological study

A section of tissue (pancreas) was carefully dissected out and fixed in 10% formal saline and processed. After fixation tissues were embedded in paraffin. Fixed tissues were cut at 5µm and stained with haematoxylin and eosin. The stained sections were examined under microscope and photographs were taken. The histological changes were recorded with the help of a pathologist.

2.9 Statistical analysis

The mean, standard deviation (SD) and probability test (Analysis of variance - ANOVA) were carried out according to Steel and Torrie [32] using BASIC programming techniques on SPSS PC for different parameters. The p value of more than 0.05 was considered as not significant.

3. Results

3.1 Biochemical analysis

Figure 1 illustrates the blood glucose levels of control and experimental rats on 0, 1st 2nd, 3rd and 4th week after drug or CALEt treatment. There was significant reduction in the blood glucose level in CALEt or glibenclamide treated diabetic rats. A gradual glucose lowering effect was seen in all the treated rats and the maximum response was observed after 4 weeks treatment period. No significant change was observed in a the blood glucose levels in normal rats treated with CALEt.

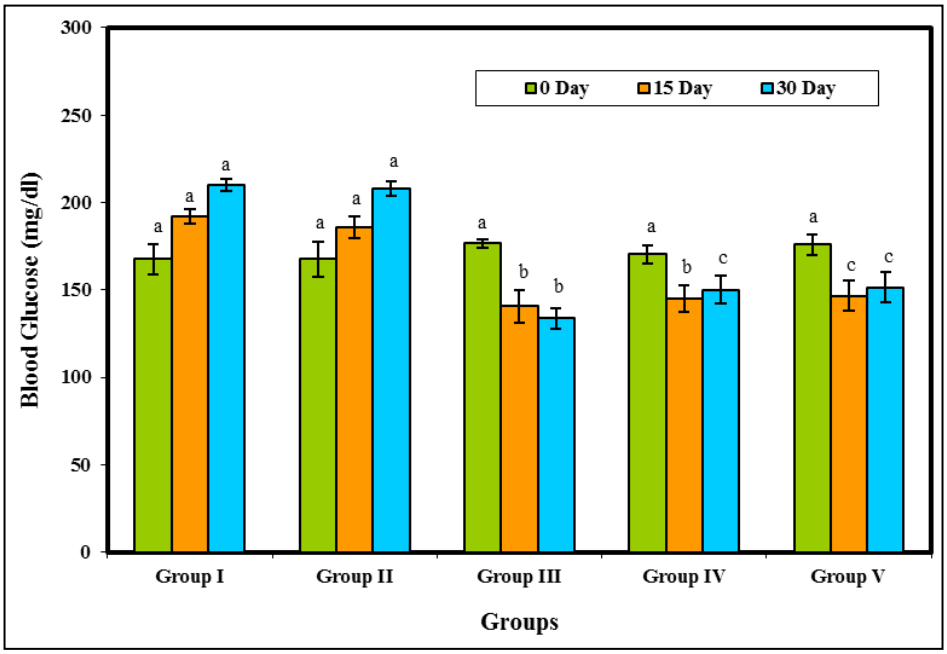

Fig 1: Effect of CAL ET on blood glucose levels in the control and experiment group of rats

Values are mean ± S.D. of 6 individual rats; Mean values that do not share same superscript differ significantly from each other at $P< 0.05$ Figure 2 depicts the body weights of control and experimental rats on 0, 15 and 30days after drug or CALEt treatment. Body weights were significantly ($P< 0.001$) decreased from day 1 to day 30 in the diabetic untreated rats. The decreased body weights in diabetic rats were significantly regained, on receiving CALEt and glibenclamide treatment.

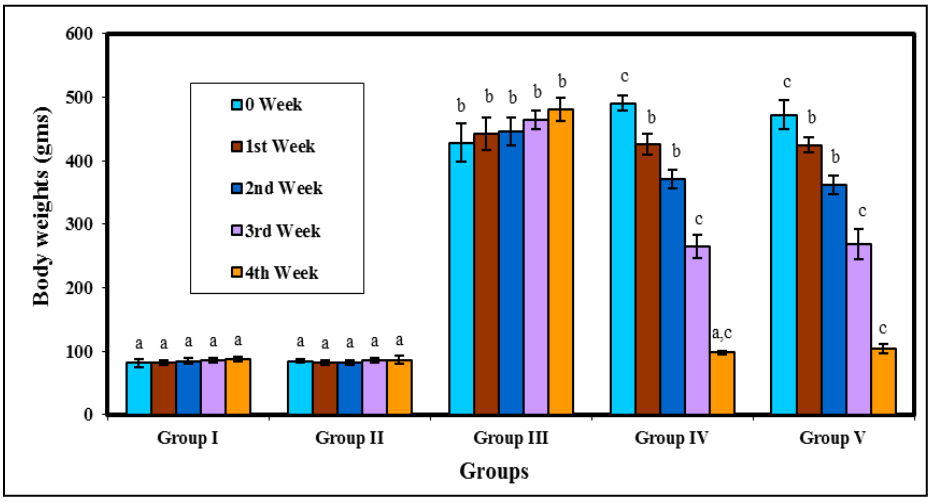

Fig 2: Effect of CALEt on body weights in the control and experiment group of rats

Values are mean ± S.D. of 6 individual rats; Mean values that do not share same superscript differ significantly from each other at $P< 0.05$

Table Ire present the levels of insulin, glucagon and glycosylated haemoglobin in control and experimental rats. A significant decrease in insulin level with significant increase in glucagon and glycosylated haemoglobin was observed diabetic rats which were reverted to near normal by administration of CALEt and glibenclamide to the diabetic rats (Group IV and Group V).

Table 1: Effect of CALEt on Insulin and Glucagon levels in the Control and Experimental Group of Rats after 30 Days Treatment (Mean ± SD)

Parameters	Group I	Group II	Group III	Group IV	Group V
Insulin (µg/ml)	5.9 1± 0.3 ^{a}	6.12 ± 0.4 ^{a}	3.11 ± 0.1^{b}	5.74 ± 0.1^{c}	5.77 ± 0.2 ^{c}
Glucagon (pg/mol)	35.8 ± 4.6^{a}	34.7 ± 3.9 ^{a}	53.7 ± 8.8^{b}	39.7 ± 3.7^{c}	38.9 ± 6.8^{c}
HbA1C (% Hb)	5.66±0.62^{a}	6.47± 1.2^{a}	11.22±1.1^{b}	6.88± 0.8^{c}	6.71±0.51^{c}

Values are given as mean± S.D of six individuals; Mean values that do not share same superscript differ significantly from each other at $P< 0.05$ Table II represents the changes in RBC count, WBC count and total haemoglobin in control and experimental rats. In diabetic rats there was a significant decrease in RBC count, WBC count and total haemoglobin. Oral administration of CALEt and glibenclamide to the diabetic rats (Group IV and Group V) significantly restored to near normal.

Table 2: Effect of CALEt on Hematology in the Control and Experimental Group of Rats after 30 Days Treatment (Mean ± SD)

Parameters	Group I	Group II	Group III	Group IV	Group V
Hemoglobin (mg/dl)	10.2±0.14[a]	10.3±0.14[a]	7.0±0.14[b]	9.7±0.13[c]	9.6±0.10[c]
RBC (Million/cumm)	4.8±0.13[a]	4.8±0.17[a]	2.3±0.10[b]	4.4±0.14[c]	4.2±0.11[c]
WBC (Thousand/cumm)	5.8±0.10[a]	5.9±0.14[a]	3.2±0.17[b]	5.5±0.18[c]	5.3±0.16[c]

Values are given as mean ± S.D of six individuals; Mean values that do not share same superscript differ significantly from each other at $P < 0.05$

3.2 Histopathology

In the pancreas of group I (Normal control rats) and group II (Normal rats treated with CALEt), many round and elongated islets were evenly distributed throughout the cytoplasm, with their nucleus lightly stained than the surrounding acinar cells (Fig 3. and Fig 4). We can see the damaged β cells, damaged islets shrunken in size and infiltration of lymphocytes and damaged β cells were observed in group III (diabetic untreated) rats (Fig 5). In group IV and group V rats that are treated with CALEt and glibenclamide, islets and β cells were considerably comparable to normal rats (Fig 6 and Fig 7).

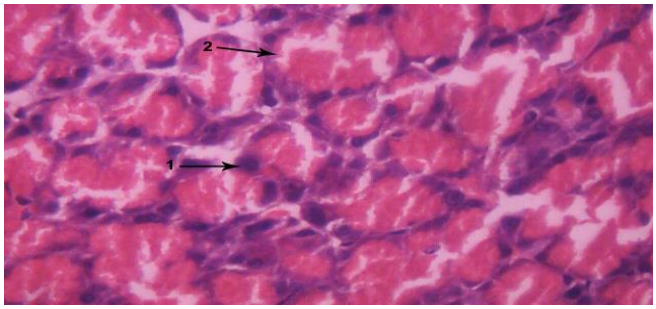

Fig 3: Control rats (Group I) pancreas showing 1. Normal islets of Langerhans 2. β-cells in H & E. 40 X magnification

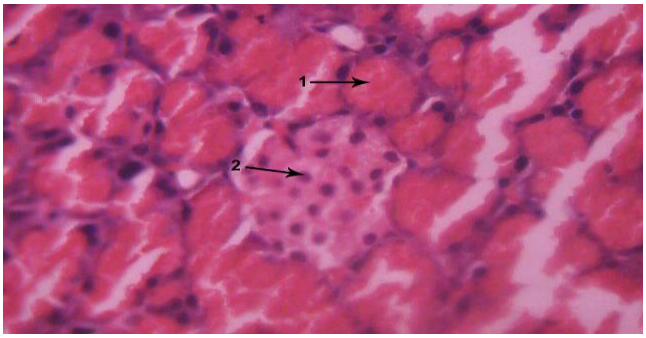

Fig 4: Normal rats treated with *Centella asiatica* (Group II) pancreas showing 1. Normal islets of Langerhans 2. β-cells in H & E 40 X magnification

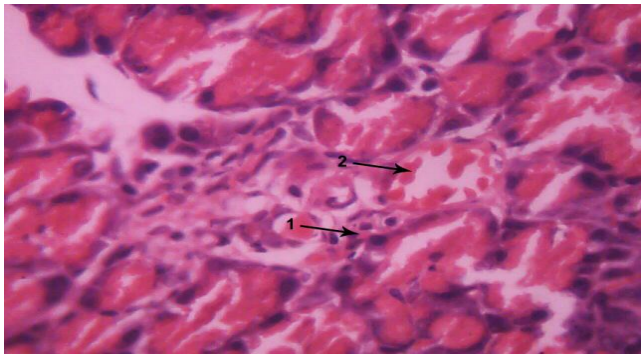

Fig 5: Diabetic untreated rats (Group III) pancreas showing 1. Destruction of β-cells and 2. Small and shrunken islets in H & E. 40 X magnification

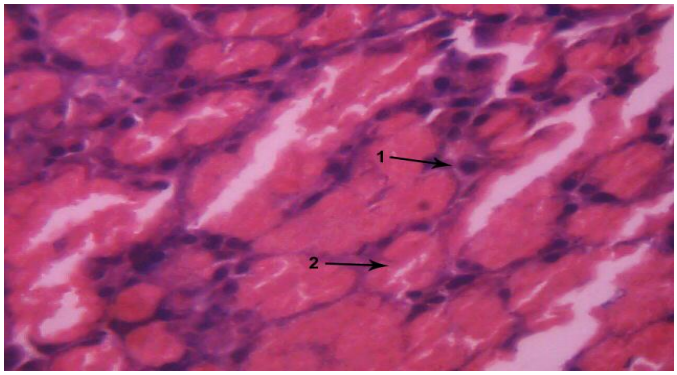

Fig 6: Diabetic rats treated with *Centella asiatica* (Group IV) pancreas showing 1. Normal islets of Langerhans 2. β-cells in H & E. 40 X magnification.

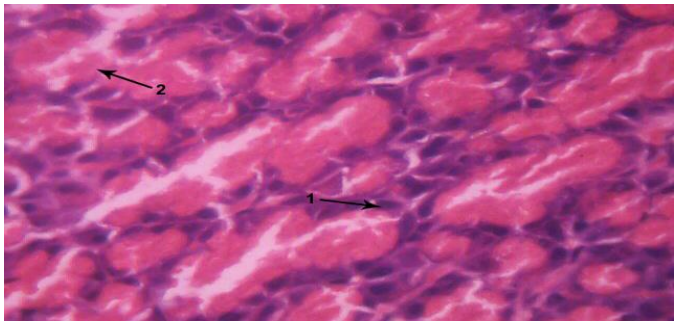

Fig 7: Diabetic rats treated with Glibenclamide (Group V) pancreas showing 1. Mild recovery of β-cells 2. Abundant patches of β-cells in pancreas in H & E. 40 X magnification.

4. Discussion

Streptozotocin naturally occurs as abroad-spectrum antibiotic, and cytotoxic chemical that is principally toxic to the pancreatic, insulin producing β-cells [33, 34]. Streptozotocin has been widely used to induce type I diabetes in animal models especially in rats and mice [35]. Streptozotocin injection leads to the degeneration of the β-cells [34, 36]. It is renowned that streptozotocin destroys insulin secreting β-cells in the islets of Langerhans and its effect is irrevocable [37]. In the current study, decreased body weight observed in diabetic untreated rats in comparison to normal rats point out that loss of body weight is a consequence of excessive break down of tissue proteins which is a distinctive condition of diabetics [38]. Treatment with CALEt improved body weight to certain extent. The significant gain in body weight in the treated rats may be due to its' protective effect in controlling muscle wasting i.e. reversal gluconeogenesis and glycogenolysis [39]. This study revealed that CALEt produced a marked decrease in blood glucose level in streptozotocin induced diabetic rats after 30 days treatment. In addition, glucose levels are restored to almost normal glycemic state. It is also pertinent to note that normal rats treated with CALEt did not show any significant change in blood glucose level after treatment, suggesting that antidiabetic property of CALEt is confined to diabetes only without affecting glucose metabolism. The antidiabetic effect of CALEt may be due to the increased release of the insulin from the existing/regenerated β cells of pancreas. Further, the anti-hyperglycemic activity of CALEt was associated with an increase in serum insulin level

suggesting an insulin genic activity of the extract. In the literature many plants have been reported to contain anti-hyperglycemic and insulin-release stimulatory effect [40–42]. Interestingly, our data even hint that antidiabetic action of CALEt has been attributed to stimulation of insulin release from the pancreatic β-cells and inhibition of glucagon secretion from pancreatic α-cells, because regular administration of this extract had positive effect on serum insulin level and negative effect on serum glucagon level in diabetic rats. Few investigators reported that there was selective destruction of pancreatic islet cells in STZ induced diabetic model, since some cells do survive, and the insulin secretion can be provoked in the enduring β-cells of the diabetic animals [43, 44]. Moreover, the present study also reveals that the destruction of cells appears to be partially and not completely, because serum insulin levels in the diabetic rats was about 52% of that in normal rats. The observed increase in the insulin level indicates that CALEt stimulates insulin secretion from the remnant or/and regenerated β-cells. The ultra-structure of diabetic pancreas showed substantial reduction in the islets of langerhans, and depleted islets. Signs of regeneration of β-cells have been reported following consumption of CALEt extract. The regeneration of the pancreas of the destructed islets is probably due to the truth that pancreas encompasses stable cells, which have the capacity of regeneration. However, it is difficult to predict the exact percentage of regeneration that has taken place after treatment. In the current study, diabetic rats had shown the elevated levels of HbA1C and decreased level of total hemoglobin indicating the poor glycemic control. Elevated levels of glycosylated hemoglobin are known to be coupled with diabetes mellitus. In general, in the non-diabetics' glycosylated hemoglobin is present at a level of about 5% of total hemoglobin while in diabetics it will be about 2-4 times more than that of normal percentage [45]. CALEt treated diabetic rats significantly decreased the HbA1C level and increased the total haemoglobin level, which might be the result of improved glucose metabolism. In this study the observed decreased levels of total haemoglobin and RBC count in diabetic rats gives an indication of anemic condition. The available literature reveals that anemia occurrence in diabetics is due to the increased non-enzymatic glycosylation of RBC membrane proteins, which correlates with hyperglycemia [46]. Oxidation of the RBC membrane proteins and hyperglycemia in diabetes mellitus grounds for the increased production of lipid peroxides that show the way to haemolysis of RBC [47]. CALEt treatment improved RBC level from diabetic reduced state. Streptozotocin is awell-recognized drug to suppress the immune system by damaging white blood cells and certain organs in the body [48]. In the current study, WBC count was significantly reduced in the streptozotocin induced diabetic untreated rats. The diminution of WBC count could be associated to suppression of leukocytosis from the bone marrow which may possibly account for deprived protective mechanisms against infection [49]. The white blood count was significantly restored to near normal after the CALEt treatment. The most important finding of this investigation is that the histopathological investigation along with the biochemical evaluations demonstrated that a pancreatic lesion induced by streptozotocin leading to full-blown diabetes was reversed upon CALEt treatment.

5. Conclusion

This study reveals that methanol extract of *Centella asiatica* leaves has potential anti-diabetic action against streptozotocin induced diabetic rats, and the effect was almost parallel to the reference drug glibenclamide. These results evidently support the traditional use of *Centella asiatica* in treatment of diabetes mellitus. However, systematic studies in humans are also needed to identify the effect and mechanism of methanol extract of *Centella asiatica* leaves.

6. Acknowledgement: None

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