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Anti-diabetic Activity of Different Solvent Extracts of *Dactyloctenium aegyptium* in Streptozotocin Induced Diabetic Rats.

Nagarjuna S[1]*, Gopala Krishna Murthy TE[2], and Srinivasa Rao A[3].

[1]Division of Pharmacology, Raghavendra Institute of Pharmaceutical Education and Research, Krishnam Reddy Palli cross, Chiyyedu, Anantapuramu-515721, Andhra Pradesh, India.
[2]Division of Pharmaceutics, Bapatla College of Pharmacy, Bapatla, Guntur-522101, Andhra Pradesh, India.
[3]Division of Pharmacology, Bhaskara Pharmacy College, Moinabad, Hyderabad-501504, Telangana, India.

ABSTRACT

Diabetes mellitus is a chronic metabolic disorder characterized by high levels of glucose in the blood due to the impaired secretion of insulin or insulin insensitivity. Despite lack of scientific evidences to support its therapeutic efficacy, the use of herbal supplements has signficantly increased. Present study was designed to investigate antidiabetic potential of different solvent extracts of *Dactyloctenium aegyptium* whole plant in streptozotocin induced diabetic rats. Six solvent extracts viz. aqueous, hydroalcoholic, ethanolic, ethyl acetate, chloroform and n-Hexane extracts were prepared by cold maceration. These extracts shown qualitative difference in phytochemical constituents. Among the extracts tested, EDA shown more significant antidiabetic activity and antidiabetic potency was in the order of EDA>HADA>ADA>EADA>CDA>NHDA. So, EDA was investigated further for its action on insulin, Hb, HbA1c, oxidative parameters, body weight and cell integrity of pancreas. Results indicated that animals treated with EDA shown significant decrease in blood glucose, HbA1c, malondialdehyde levels and significant increase in insulin, Hb, SOD, catalase, reduced glutathione and body weight. It could be concluded that *Dactyloctenium aegyptium* might be used in the treatment of diabetes , however necessary studies on characterization of active principles and their mode of action are required for use of this plant as antidiabetic agent.

Keywords: *Dactyloctenium aegyptium,* antidiabetic activity, different solvent extracts, streptozotocin.

Corresponding author

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INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder affecting a major proportion of the population worldwide. It is characterized by hyperglycaemia and associated with an absolute or relative deficiency in the insulin action or secretion. It is a growing health problem in most countries and its incidence is considered to be high (4%-5%) all over the world [1]. There are an estimated 143 million people in the world with diabetes mellitus and this number will probably double by the year 2030. Chronic hyperglycemia causes complications linked to diabetes, such as heart disease, retinopathy, kidney disease and neuropathy. It is also a common cause of chronic morbidity and disability among the working population in the world. Streptozotocin induces its diabetogenic activity mainly by inducing oxygen free radicals, thereby damaging the pancreas [2]. Supplementation with non-toxic free radical scavengers and antioxidants may facilitate the regeneration of β-cells and protect pancreatic islets against the cytotoxic effects of streptozotocin [3]. Marketed preparations of oral hypoglycaemic agents exhibit several side effects. Thus, there is a need for more effective oral antihyperglycaemic agents, particularly those that normalise both insulin and glucose levels. Moreover, the plant kingdom represents a largely unexplored reservoir of biologically active compounds, which as well as being developed into drugs, could provide lead compounds for the synthesis of bioactive analogues[4]. *Dactyloctenium aegyptium* belongs to family Poaceae (Gramineae), commonly known as Nela raagi in telugu and it is widely distributed all over India and other parts of Asia. Of the several plants used in the treatment of Diabetes mellitus, *Dactyloctenium aegyptium* is one of those plants used to treat diabetes, worm infections, wounds, pains, kidney diseases etc. in folklore medicine. Literature survey has shown that there are no scientific reports available on the effects of *Dactyloctenium aegyptium* on diabetes mellitus. So the present study was planned to explore the antihyperglycemic potential of different solvent extracts of *Dactyloctenium aegyptium* whole plant in streptozotocin induced diabetic rats.

MATERIALS AND METHODS

Plant Material

For the present study, *Dactyloctenium aegyptium* whole plant was collected from the forest area near to the Madanapalli of Chittoor district of Andhra Pradesh and the plant was botanically identified and authenticated by Dr. K. Madhava Chetty, Assistant professor, Department of Botany, S.V. University, Tirupati, A.P., India and a voucher specimen (RIPER/SN/002) was preserved in division of pharmacology, RIPER, Anantapur for further reference.

Preparation of Plant Extracts

Collected plant material was thoroughly examined for the foreign material, washed with water and shade dried for 21 days then made into powder by mechanical grinder. Extraction was carried out by cold maceration method using different solvents like water, hydroalcohol (water: methanol 50:50), ethanol, ethyl acetate, chloroform and n- hexane for 72 hours. Then the contents were filtered and the filtrates were concentrated using rotary flash evaporator, calculated for their yield and stored in desiccators till further use.

Preliminary Phytochemical Screening

Freshly prepared crude extracts were qualitatively tested for the presence of chemical constituents. Phytochemical screening of the extracts was performed and these were identified by characteristic colour changes using standard procedure.

Drugs and Chemicals

Streptozotocin was procured from Sigma Aldrich Labs, Gliclazide was provided as a gift sample from Dr. Reddy's Laboratories, Glucose kits were procured from Erba diagnostics.

Experimental Animals

Animals were housed in plastic cages (28 cm×43 cm×18 cm) and were maintained under conventional laboratory conditions (temperature 22±2^{0}C and humidity 50±15%) with a regular 12-h light/12-h dark cycle

throughout the study. They were fed standard pellet chow and were allowed water *ad libitum*. Wistar rats of both sexes weighing 150-200gm were used for the study. All protocols were performed in accordance with the Institutional Animal Ethical Committee (IAEC) of RIPER as per the directions of the CPCSEA (Committee for the purpose of Control and Supervision on Experiments on Animals).

Acute Toxicity Studies

Acute oral toxicity study was performed as per Organization for Economic Cooperation and Development (OECD) guidelines 423. Swiss albino mice were administered with different solvent extracts up to 2000mg/kg. Animals were observed individually for gross behavioural changes as well as for mortality for 14 days.

Induction of Diabetes

After fasting for 18 h, diabetes was induced by intraperitoneal injection of streptozotocin dissolved in 0.1 M cold sodium citrate buffer, pH 4.5, at a dose of 55 mg/kg [5]. The animals were allowed to drink 5% glucose solution overnight to overcome the drug induced hypoglycaemia [6]. After 72 h, rats with marked hyperglycemia (FBG ≥250 mg/dl) were selected and used for the study. All the animals were allowed free access to tap water and pellet diet and maintained at room temperature in plastic cages.

Experimental Design

Effect of different solvent extracts of *Dactyloctenium aegyptium* on serum glucose levels in streptozotocin induced diabetic rats

The animals were divided into nine groups and each group consisted of six rats.

Group I: Untreated normal rats
Group II: Untreated diabetic rats
Group III: Diabetic rats treated with Gliclazide 4.5mg/kg, p.o. for 30 days
Group IV: Diabetic rats treated with aqueous extract of *Dactyloctenium aegyptium* (ADA) 200mg/kg, p.o. for 30 days
Group V: Diabetic rats treated with hydroalcoholic extract of *Dactyloctenium aegyptium* (HADA) 200mg/kg, p.o. for 30 days
Group VI: Diabetic rats treated with ethanolic extract of *Dactyloctenium aegyptium* (EDA) 200mg/kg, p.o. for 30 days
Group VII: Diabetic rats treated with ethyl acetate extract of *Dactyloctenium aegyptium* (EADA) 200mg/kg, p.o. for 30 days
Group VIII: Diabetic rats treated with chloroform extract of *Dactyloctenium aegyptium* (CDA) 200mg/kg, p.o. for 30 days
Group IX: Diabetic rats treated with n-hexane extract of *Dactyloctenium aegyptium* (NHDA) 200mg/kg, p.o. for 30 days.

Blood samples were collected on 1st, 10th, 20th and 30th day of study from retro orbital venous plexus following the technique described by Coccheto and Bjornsson [7], allowed to clot and were centrifuged at 3000 rpm for 20 min. The serum was separated and used for the estimation of glucose levels [8] .

Effect of EDA on serum insulin levels

On 31st day of experiment, blood samples were collected by cardiac puncture from group I, II, III and VI and allowed to clot and were centrifuged at 3000 rpm for 20 min. The serum was separated and used for the estimation of insulin [9] levels.

Effect of EDA on blood Hb and glycosylated haemoglobin (HbA1c) levels

On 31st day of experiment, blood samples were collected by cardiac puncture from group I, II, III and VI and used for the estimation of Hb and HbA1c[10] levels.

Effect of EDA on serum oxidative parameters

On 31st day of experiment, blood samples were collected by cardiac puncture from group I, II, III and VI and allowed to clot and were centrifuged at 3000 rpm for 20 min. The serum was separated and used for the estimation of SOD [11], catalase, reduced glutathione [12] and malon dialdehyde [13] (MDA).

Effect of EDA on body weight

Change in body weight of animals from group I, II, III and VI was determined by weighing the animals on day 1 and 30.

Histopathological Procedures

On 31st day of experiment, group I, II, III and VI rats were sacrificed under anaesthesia, pancreas were immediately excised, fixed in 10% solution of formaldehyde and histopathological studies were carried out at Star diagnostic laboratories, Anantapuramu, A.P., India.

Statistical Analysis

Data were expressed as mean ± standard error of the mean (S.E.M); and comparison between the different treatments was carried out using analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test using computerized Graph Pad Prism, version 4.5 software (Graph Pad Software Inc).

RESULTS

Percentage yield, colour and nature of different solvent extracts of *Dactyloctenium aegyptium*

The results are tabulated in table number 1.For the present study extraction was carried out by cold maceration method using different solvents and all the extracts were evaluated for their percentage yield, colour and nature.

Table 1: Percentage yield, colour and nature of different solvent extracts of *Dactyloctenium aegyptium*

S. No.	Extract	% yield	Colour	Nature
1	ADA	14%	Dark brown	Semisolid
2	HADA	20%	Dark brown	Semisolid
3	EDA	25%	Dark brown	Semisolid
4	EADA	6%	Dark brown	Semisolid
5	CDA	4.3%	Brown	Semisolid
6	NHDA	3.8%	Brown	Semisolid

Preliminary phytochemical screening

The results are tabulated in table number 2. Aqueous extract revealed the presence of carbohydrates, proteins, amino acids, saponins, flavonoids and tannins. Hydroalcoholic extract revealed the presence of carbohydrates, proteins, amino acids, saponins, flavonoids, tannins, terpenoids and alkaloids. Ethanolic extract revealed the presence of carbohydrates, proteins, amino acids, saponins, flavonoids, tannins, terpenoids and alkaloids. Ethyl acetate extract revealed the presence flavonoids, tannins, terpenoids and alkaloids. Chloroform and n- hexane extracts revealed the presence of terpenoids.

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Table 2: Preliminary phytochemical screening

S.No.	Constituents	ADA	HADA	EDA	EADA	CDA	NHDA
1.	Carbohydrates	+ve	+ve	+ve	-ve	-ve	-ve
2.	Proteins	+ve	+ve	+ve	-ve	-ve	-ve
3.	Amino acids	+ve	+ve	+ve	-ve	-ve	-ve
4.	Anthraquinones	-ve	-ve	-ve	-ve	-ve	-ve
5.	Saponins	+ve	+ve	+ve	-ve	-ve	-ve
6.	Flavonoids	+ve	+ve	+ve	+ve	-ve	-ve
7.	Tannins	+ve	+ve	+ve	+ve	-ve	-ve
8.	Terpenoids	-ve	+ve	+ve	+ve	+ve	+ve
9.	Steroids	-ve	-ve	-ve	-ve	-ve	-ve
10.	Cardiac glycosides	-ve	-ve	-ve	-ve	-ve	-ve
11.	Fats & oils	-ve	-ve	-ve	-ve	-ve	-ve
12.	Alkaloids	-ve	+ve	+ve	+ve	-ve	-ve
13.	Cyanogenic Glycosides	-ve	-ve	-ve	-ve	-ve	-ve
14.	Coumarin Glycosides	-ve	-ve	-ve	-ve	-ve	-ve

Acute toxicity studies

For all extracts, no toxicity was found up to 2000 mg/kg. Hence, 1/10th (200mg/kg b.wt.) was selected for further studies.

Effect of different solvent extracts of *Dactyloctenium aegyptium* on serum glucose levels in streptozotocin induced diabetic rats

The results are tabulated in table number 3. All extracts under study shown significant decrease in serum glucose levels and antidiabetic potency of extracts was in the order of EDA>HADA>ADA>EADA>CDA>NHDA. So based upon the yield and effect on serum glucose levels, EDA was selected for further investigations.

Table 3: Effect of different solvent extracts of *Dactyloctenium aegyptium* on serum glucose levels in streptozotocin induced diabetic rats

S. No.	Group	Serum glucose levels (mg/dL)			
		1st Day	10th Day	20th Day	30th Day
1	Normal	93.52±1.243	90.20±0.844	90.85±1.891	90.18±3.934
2	Diabetic control	350.5±6.449$^{\#}$	434.5±7.455$^{\#}$	451.5±4.992$^{\#}$	473.5±2.754$^{\#}$
3	Standard	346.5±4.052^{ns}	171.5±5.123*	139.3±2.869*	96.75±1.887*
4	ADA	353.5±1.708^{ns}	203.5±2.986*	185.0±2.646*	143.5±3.096*
5	HADA	347.0±5.431^{ns}	198.8±2.926*	166.3±2.529*	116.5±4.500*
6	EDA	346.5±6.397^{ns}	184.8±4.230*	154.3±3.065*	95.75±2.594*
7	EADA	342.0±4.397^{ns}	229.8±4.328*	208.8±2.689*	168.8±5.851*
8	CDA	350.0±6.055^{ns}	232.5±3.797*	212.8±2.562*	176.3±4.404*
9	NHDA	351.3±3.250^{ns}	260.8±24.87*	218.8±3.351*	181.8±3.838*

Values are expressed as mean ± S.E.M, n=6 in each group. #p <0.001 when compared to normal, ns- nonsignificant when compared to diabetic control *p< 0.001 when compared to diabetic control.

Effect of EDA on serum insulin levels

The results are graphically illustrated in Fig.1.Streptozotocin significantly decreased serum insulin levels when compared to normal group. Administration of EDA significantly increased serum insulin levels when compared to diabetic group.

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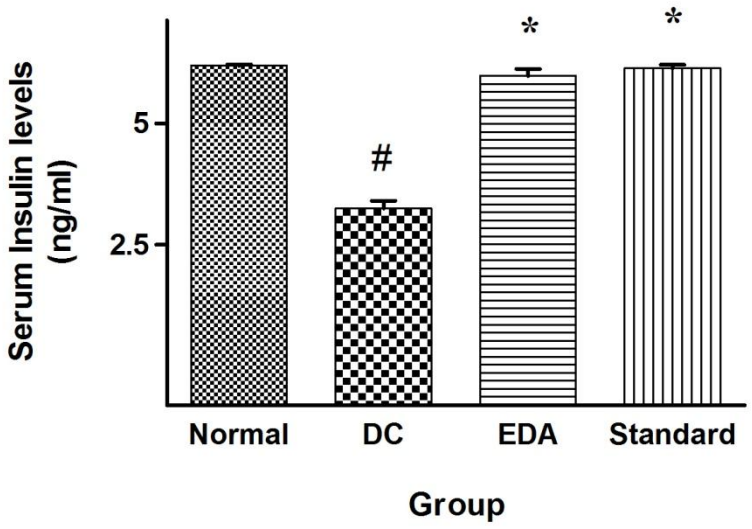

Figure 1: Effect of EDA on serum insulin levels

Values are expressed as mean ± S.E.M, n=6 in each group#p <0.001 when compared to normal, *p< 0.001 when compared to diabetic control.

Effect of EDA on blood Hb and glycosylated haemoglobin (HbA1c) levels

The results are graphically illustrated in Fig.2. Streptozotocin significantly decreased blood Hb levels and increased HbA1c levels when compared to normal group. Administration of EDA significantly increased blood Hb levels and decreased HbA1c levels when compared to diabetic group.

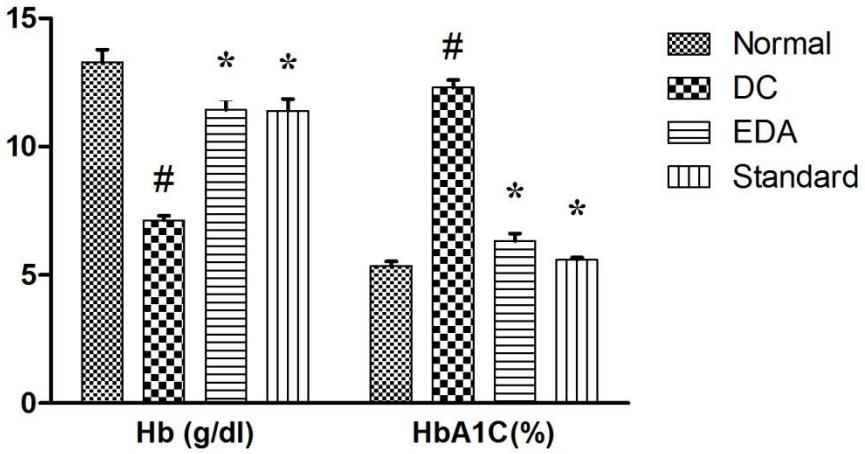

Figure 2: Effect of EDA on blood Hb and glycosylated haemoglobin (HbA1c) levels

Values are expressed as mean ± S.E.M, n=6 in each group. #p <0.001 when compared to normal, *p< 0.001 when compared to diabetic control.

Effect of EDA on serum oxidative parameters

The results are graphically illustrated in Fig.3. Streptozotocin significantly decreased serum SOD, catalase, GSH and significantly increased malondialdehyde levels when compared to normal group. Administration of EDA significantly increased serum SOD, catalase, GSH and significantly decreased malondialdehyde levels when compared to diabetic group.

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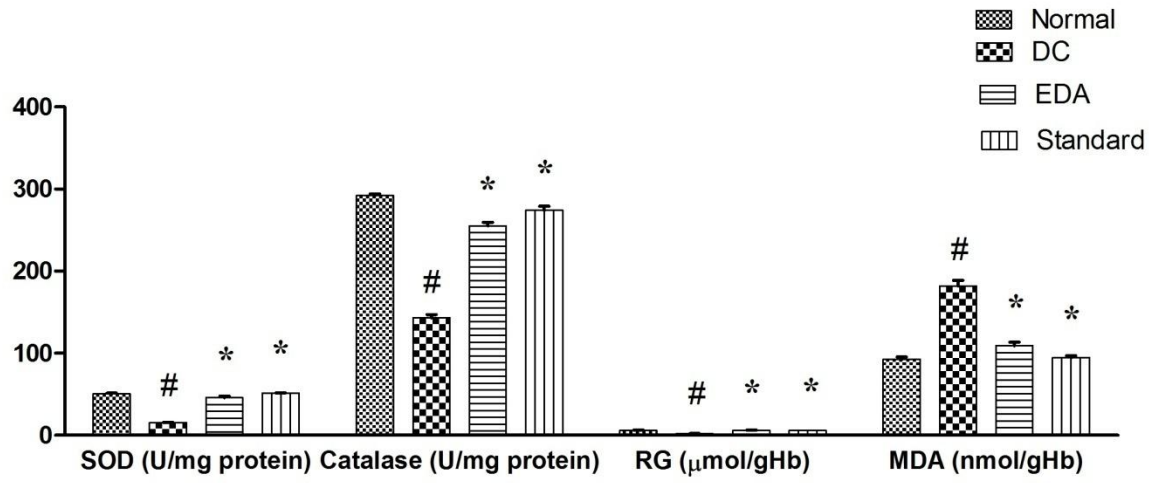

Figure 3: Effect of EDA on serum oxidative parameters

Values are expressed as mean ± S.E.M, n=6 in each group, #p <0.001 when compared to normal, *p< 0.001 when compared to diabetic control.

Effect of EDA on body weight

The results are graphically illustrated in Fig.4. Administration of EDA to diabetic rats resulted in increased body weight compared to untreated diabetic rats.

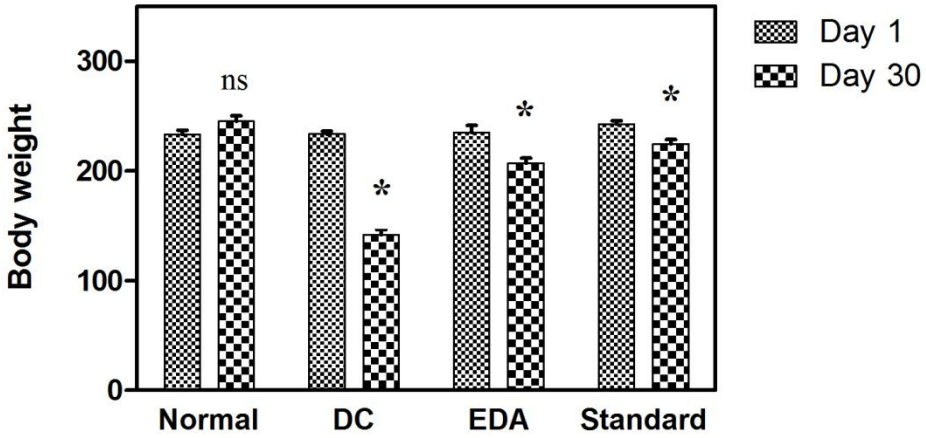

Figure 4: Effect of EDA on body weight

ns- nonsignificant, *p< 0.001 when compared to day 1.

Histopathological studies

The cellular integrity and architecture were intact in normal group (figure 5A). Whereas diabetic animals showed marked acinar atrophy and the acinar architecture was destroyed (figure 5B). In case of EDA and Gliclazide treated group, the pancreas appears almost normal when compared to diabetic rats (figure 5C&5D).

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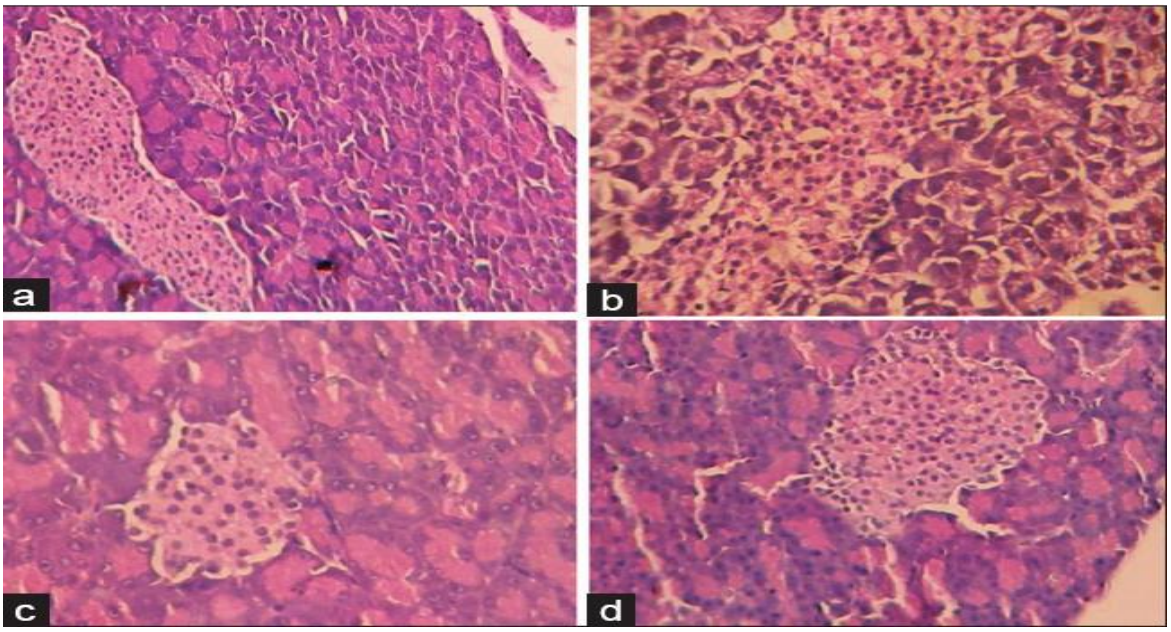

Figure 5: Effect of EDA on cell integrity of pancreas

a: Normal; b: Diabetic control; c: EDA and d: Gliclazide

DISCUSSION

People on all continents have used hundreds to thousands of indigenous plants for treatment of ailments since prehistoric times. According to WHO, about 80% of the world's population presently uses phytotherapy for some aspect of primary health care system. There are many pharmaceutical products which are available in modern medical treatment have a long history of use as herbal remedies including aspirin, opium, digitalis and quinine [14]. A large number of world's population who live in developing countries cannot take the benefits of modern pharmaceuticals as those are very expensive. Hence, phytotherapy is still a popular means of primary healthcare for which people bear a little or no cost. In addition to the use in the developing world, phytotherapy is used in the industrialized nations by alternative medicine practitioners such as naturopaths. Among the 120 active compounds currently isolated from the higher plants and widely used in modern medicine today, 80% show a positive correlation between their modern therapeutic use and the traditional use of the plants from which they are derived [15]. Approximately 25% of modern drugs used in the United States have been derived from plant origins [14]. So, research on phytotherapy has got great momentum in recent years to find out noble pharmaceuticals.

In the present study, streptozotocin was chosen to induce diabetes in rats because of its lower toxicity and higher β-cell specificity relative to other diabetogens [16]. Findings of the present investigation revealed that STZ induced diabetes resulted in a significant increase in serum glucose levels, decrease in serum insulin levels, decrease in Hb levels and elevation in glycosylated haemoglobin (HbA1c) levels. Furthermore STZ significantly increased oxidative stress indicated by decrease in serum SOD, catalase, GSH and increase in serum MDA levels. In light of the results, our study indicates that *Dactyloctenium aegyptium* whole plant has good antidiabetic activity. Data of current study showed that different solvent extracts exhibited antihyperglycemic activity but EDA shown more significant effect on blood glucose levels. Hence, it was selected for further investigation on other biochemical parameters. Data of present investigation revealed that daily administration of EDA for 30 days reduced hyperglycemia which is evidenced by significant reduction in glucose levels; serum HbA1c levels as well as significant rise in serum insulin and serum Hb levels. Furthermore significant increase in serum SOD, catalase, GSH and significant decrease in serum MDA levels were observed in animals treated with EDA for 30 days when compared to diabetic control group. These reports also support the histopathological observations wherein both the cellular integrity and architecture were restored towards normalization after treatment with EDA.

CONCLUSION

The present study reports for the first time to our knowledge that *Dactyloctenium aegyptium* possesses antidiabetic activity. Observed antidiabetic and antioxidant activity of the title plant may be attributed to bioactive flavonoids. The major outcome of the study is to provide a platform for the application of whole plant of *Dactyloctenium aegyptium* or active antidiabetic constituent of *Dactyloctenium aegyptium*, either to supplement existing oral antidiabetic drugs for the treatment of diabetes or to reduce the transformation of prediabetics into diabetics.

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