



## Antidiabetic Activity of Different Fractions of Ethanollic Extract of *Dactyloctenium aegyptium* in Streptozotocin Induced Diabetic Rats

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### Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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### ABSTRACT

**Aims:** The present study was aimed to evaluate the antidiabetic effect of *n-hexane*, Chloroform, Ethyl acetate and Methanolic fractions from ethanollic extract of *Dactyloctenium aegyptium* in streptozotocin induced diabetic rats.

**Study Design:** The animals were divided into seven groups and each group consisted of six rats.

**Place and Duration of Study:** Laboratory of Raghavendra Institute of Pharmaceutical Education & Research (RIPER), Anantapuramu and 35 days.

**Methodology:** The animals were divided into seven 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 4.5 mg/kg, p.o. for 30 days, Group IV- Diabetic rats treated with *n-hexane* fraction of

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EDA (NHF) 50 mg/kg, p.o. for 30 days, Group V- Diabetic rats treated with Chloroform fraction of EDA (CF) 50 mg/kg, p.o. for 30 days, Group VI: Diabetic rats treated with Ethyl acetate fraction of EDA (EAF) 50 mg/kg, p.o. for 30 days, Group VII: Diabetic rats treated with Methanolic fraction of EDA (MF) 50 mg/kg, p.o. for 30 days and the serum was separated and used for the estimation of glucose levels. On 31<sup>st</sup> day of experiment, best fraction was investigated further for its action on insulin, Hb, HbA1c, oxidative parameters, body weight and cell integrity of pancreas.

**Results:** Results indicated that animals treated with MF shown significant decrease in blood glucose, HbA1c, malondialdehyde levels and significant increase in insulin, Hb, SOD, catalase, reduced glutathione and body weight.

**Conclusion:** It could be concluded that methanolic fraction of ethanolic extract of *Dactyloctenium aegyptium* has favourable effect in bringing down the severity of diabetes however necessary studies are required on characterization of active principles.

**Keywords:** *Dactyloctenium aegyptium*; streptozotocin; antidiabetic activity; fractions; glucose.

## 1. INTRODUCTION

Diabetes mellitus (DM) is a chronic disorder caused by inherited and/or acquired deficiency in production of insulin by the pancreas or by the ineffectiveness of the insulin produced. Diabetes is divided into two major categories: Type 1 diabetes [Insulin dependent diabetes mellitus (IDDM)] and Type 2 diabetes [Non-insulin dependent diabetes mellitus (NIDDM)]. Type 1 diabetes, the cause is an absolute deficiency of insulin secretion and the cause of Type 2 diabetes is a combination of resistance to insulin action and an inadequate compensatory insulin secretory response [1]. According to the World Health Organization (WHO), at least 171 million people worldwide suffer from diabetes. This figure is likely to be more than double by 2030 to reach 366 million. It is estimated that about 79.4 million people in India will be diabetic by 2030. The increasing worldwide incidence of DM in adults constitutes a significant impact on the health, quality of life and life expectancy of patients, as well as on the global public health care system [2]. Currently, the available therapy for diabetes includes insulin and various oral antidiabetic agents such as sulfonylureas, thiazolidinediones,  $\alpha$ -glucosidase inhibitors etc. These drugs are used as monotherapy or in combination to achieve better glycemic control. Each of the above oral antidiabetic agents are associated with a number of serious adverse effects [3]. Therefore, the search for more effective and safer hypoglycemic agents has continued to be an important area of investigation. Plants play a major role in the discovery of new therapeutic agents and have received much attention as sources of biologically active substances including antioxidants, hypoglycemic and hypolipidemic agents [4]. To date, over 400 traditional plants

treatment for diabetes have been reported, although only a small number of these have received scientific and medical evaluation to assess their efficacy. The WHO Expert Committee on diabetes has recommended that traditional medicinal herbs be further investigated [5]. So, as a part of the ongoing research works to find out noble antidiabetic agents, here we study the whole plant of the *Dactyloctenium aegyptium*. One such plant that is being used by the traditional practitioners to treat diabetes is *D. aegyptium* belonging to the family Poaceae (Gramineae), commonly known as Nela raagi in telugu and it is widely distributed all over India and other parts of Asia [6]. Traditionally it is also being used in the treatment of gastrointestinal ailments, worm infections, wounds, pain and kidney diseases [7]. In our earlier work we investigated the antidiabetic activity of different solvent extracts of *D. aegyptium* [8]. Of which, the most effective solvent extract found was ethanolic extract. The constituents reported in this extract are carbohydrates, proteins, amino acids, saponins, flavonoids, tannins, terpenoids and alkaloids. Therefore after knowing the most effective solvent extract, isolating the active fraction from the most effective solvent extract would be useful in the development of new drugs from plants. The standard fraction of an active extract may prove better therapeutically, less toxic and inexpensive. Keeping these facts in mind, the present study was undertaken to identify the active antidiabetic fraction of the active extract.

## 2. MATERIALS AND METHODS

### 2.1 Plant Material

For the present study, *D. aegyptium* whole plant was collected from the forest area near to the

Madanapalli, 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.

## 2.2 Preparation and Fractionation of Crude 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 maceration method using ethanol for 72 hours. Then the contents were filtered and the filtrates were concentrated using rotary flash evaporator. Thus the highly concentrated crude ethanolic extract was obtained. It was then fractionated by column chromatography using n-hexane, Chloroform, Ethyl acetate and Methanol. Column chromatography was performed by using glass column and silica gel as stationary phase. The dried fractionated extracts were then preserved in the refrigerator for the experimental use.

## 2.3 Preliminary Phytochemical Screening

Freshly prepared fractionated extracts were qualitatively tested for the presence of phytochemical constituents. Phytochemical screening was performed and these were identified by characteristic colour changes using standard procedures as described by Harborne [9], Trease and Evans [10] and Sofowara [11].

## 2.4 Drugs and Chemicals

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

## 2.5 Experimental Animals

Animals were housed in plastic cages (28 cm×43 cm×18 cm) and were maintained under conventional laboratory conditions (temperature 22±2°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 150-200 gm were used for study.

All protocols were performed in accordance with the Institutional Animal Ethical Committee (IAEC) of RIPER as per the directions of the Committee for the purpose of Control and Supervision on Experiments on Animals (CPCSEA).

## 2.6 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 [12]. The animals were allowed to drink 5% glucose solution overnight to overcome the drug induced hypoglycaemia [13]. 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.

## 2.7 Experimental Design

### 2.7.1 Effect of different fractions of ethanolic extract of *D. aegyptium* (EDA) on serum glucose levels in streptozotocin induced diabetic rats

The animals were divided into seven 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 4.5 mg/kg, p.o. for 30 days.
- Group IV: Diabetic rats treated with n-hexane fraction of EDA (NHF) 50 mg/kg, p.o. for 30 days.
- Group V : Diabetic rats treated with Chloroform fraction of EDA (CF) 50 mg/kg, p.o. for 30 days.
- Group VI : Diabetic rats treated with Ethyl acetate fraction of EDA (EAF) 50 mg/kg, p.o. for 30 days.
- Group VII: Diabetic rats treated with Methanolic fraction of EDA (MF) 50 mg/kg, p.o. for 30 days.

Blood samples were collected on 1<sup>st</sup>, 10<sup>th</sup>, 20<sup>th</sup> and 30<sup>th</sup> day of study from retro orbital venous plexus following the technique described by Cocchetto and Bjornsson [14], allowed to clot and were centrifuged at 3000 rpm for 20 min. The serum was separated and used for the estimation of glucose levels [15].

### **2.7.2 Effect of MF on serum insulin levels**

On 31<sup>st</sup> day of experiment, blood samples were collected by cardiac puncture from group I, II, III and VII and allowed to clot and were centrifuged at 3000 rpm for 20 min. The serum was separated and used for the estimation of insulin levels [16].

### **2.7.3 Effect of MF on blood Hb and glycosylated haemoglobin (HbA1c) levels**

On 31<sup>st</sup> day of experiment, blood samples were collected by cardiac puncture from group I, II, III and VII and used for the estimation of Hb and HBA1c levels [17].

### **2.7.4 Effect of MF on serum oxidative parameters**

On 31<sup>st</sup> day of experiment, blood samples were collected by cardiac puncture from group I, II, III and VII and allowed to clot and were centrifuged at 3000 rpm for 20 min. The serum was separated and used for the estimation of SOD [18], catalase, reduced glutathione [19] and malondialdehyde (MDA) [20].

### **2.7.5 Effect of MF on body weight**

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

### **2.7.6 Histopathological Procedures**

On 31<sup>st</sup> day of experiment, group I, II, III and VII 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.

## **2.8 Statistical Analysis**

Data were expressed as mean  $\pm$  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).

## **3. RESULTS**

### **3.1 Percentage Yield, Colour and Nature of Different Fractions of Ethanolic Extract of *D. aegyptium***

The results are tabulated in Table 1. For the present study fractionation was carried out by column chromatography using n-Hexane, Chloroform, Ethyl acetate and Methanol and all the fractionated extracts were evaluated for their percentage yield, colour and nature.

### **3.2 Preliminary Phytochemical Screening of Different Fractions of Ethanolic Extract of *D. aegyptium***

The results are tabulated in Table 2. n-hexane fraction revealed the presence of terpenoids. Chloroform fraction revealed the presence of terpenoids and alkaloids. Ethyl acetate fraction revealed the presence of flavonoids, tannins, terpenoids and alkaloids. Methanolic fraction revealed the presence of carbohydrates, proteins, amino acids, saponins, flavonoids, tannins and alkaloids.

### **3.3 Effect of Different Fractions of Ethanolic Extract of *D. aegyptium* on Serum Glucose Levels in Streptozotocin Induced Diabetic Rats**

The results are tabulated in Table 3. All fractionated extracts under study shown significant decrease in serum glucose levels and antidiabetic potency was in the order of MF>EAF>CF>NHF. So based upon the yield and effect on serum glucose levels, MF was selected for further investigations.

**Table 1. Percentage yield, Colour and nature of different fractions of ethanolic extract of *D. aegyptium***

<b>S. no</b>	<b>Fraction</b>	<b>% Yield</b>	<b>Colour</b>	<b>Nature</b>
1.	NHF	3.6%	Greenish brown	Semisolid
2.	CF	4.2%	Greenish brown	Semisolid
3.	EAF	5.3%	Dark brown	Semisolid
4.	MF	8.6%	Dark brown	Semisolid

**Table 2. Preliminary phytochemical screening of different fractions of ethanolic extract of *D. aegyptium***

S. no	Constituents	NHF	CF	EAF	MF
1.	Carbohydrates	-ve	-ve	-ve	+ve
2.	Proteins	-ve	-ve	-ve	+ve
3.	Amino acids	-ve	-ve	-ve	+ve
4.	Saponins	-ve	-ve	-ve	+ve
5.	Flavonoids	-ve	-ve	+ve	+ve
6.	Tannins	-ve	-ve	+ve	+ve
7.	Terpenoids	+ve	+ve	+ve	-ve
8.	Alkaloids	-ve	+ve	+ve	+ve

**Table 3. Effect of different fractions of ethanolic extract of *D. aegyptium* on serum glucose levels in streptozotocin induced diabetic rats**

S. no	Group	Serum glucose levels (mg/dL)			
		1 <sup>st</sup> Day	10 <sup>th</sup> Day	20 <sup>th</sup> Day	30 <sup>th</sup> Day
1.	Normal	92.00±4.397	87.75±5.297	90.25±4.460	91.25±4.750
2.	Diabetic control	359.3±6.6 <sup>#</sup>	433.5±3.66 <sup>#</sup>	443±3.10 <sup>#</sup>	484.5±3.96 <sup>#</sup>
3.	Gliclazide	360.8±5.96 <sup>ns</sup>	185.5±3.86 <sup>ns</sup>	150.5±2.21 <sup>**</sup>	99.75±3.17 <sup>**</sup>
4.	NHF	364±3.53 <sup>ns</sup>	416.8±6.21 <sup>ns</sup>	409.3±3.63 <sup>**</sup>	380.0±6.73 <sup>**</sup>
5.	CF	368±6.78 <sup>ns</sup>	378.5±7.45 <sup>ns</sup>	302.5±9.06 <sup>**</sup>	209.0±5.19 <sup>**</sup>
6.	EAF	365.3±8.57 <sup>ns</sup>	305.8±4.32 <sup>ns</sup>	267.8±6.98 <sup>**</sup>	148.8±5.49 <sup>**</sup>
7.	MF	363.3±7.36 <sup>ns</sup>	164.3±3.32 <sup>ns</sup>	125±2.67 <sup>**</sup>	90.75±5.977 <sup>**</sup>

Values are expressed as mean ± S.E.M, n=6 in each group.

Ns- Non significant, #p<0.001 when compared to normal, \*p<0.01 when compared to diabetic control, \*\*p<0.001 when compared to diabetic control

### 3.4 Effect of MF 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 MF significantly increased serum insulin levels when compared to diabetic group.

### 3.5 Effect of MF 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 MF significantly increased blood Hb levels and decreased HbA1c levels when compared to diabetic group.

### 3.6 Effect of MF on Serum Oxidative Parameters

The results are graphically illustrated in Figs. 3, 4, 5 and 6. Streptozotocin significantly decreased

serum SOD, Catalase, GSH and significantly increased malondialdehyde levels when compared to normal group. Administration of MF significantly increased serum SOD, Catalase, GSH and significantly decreased malondialdehyde levels when compared to diabetic group.

### 3.7 Effect of MF on Body Weight

The results are graphically illustrated in Fig. 7. Administration of MF to diabetic rats resulted in increased body weight compared to untreated diabetic rats.

### 3.8 Histopathological Studies

**Normal** - Cells of the pancreas were all present in their normal proportions. The acinar cells which stained strongly are arranged in lobules with prominent nuclei. The islet cells are seen embedded within the acinar cells and surrounded by a fine capsule.

**Diabetic control** - The islets are largely occupied by a uniform eosinophilic material and

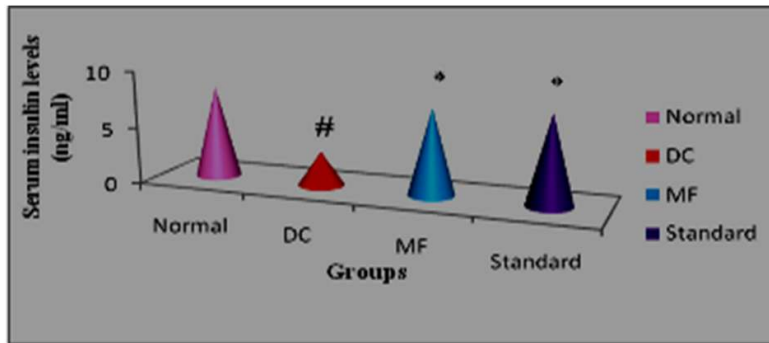
few atrophic cells. Eosinophilic materials also surround the blood vessel.

**MF** - The acinar cells & islet cells were seen to be normal.

**Gliclazide** - The acinar cells & islet cells were seen to be normal.

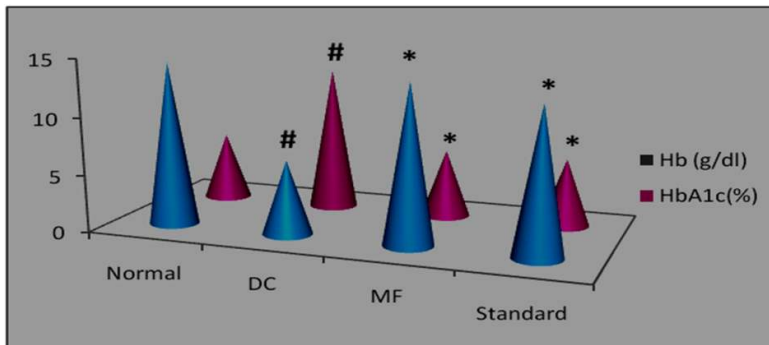
#### 4. DISCUSSION

The present study was undertaken to examine the antidiabetic activity of various fractionated extracts of ethanolic extract of *D. aegyptium* and to find out the active antihyperglycemic fraction of the active extract of this plant.



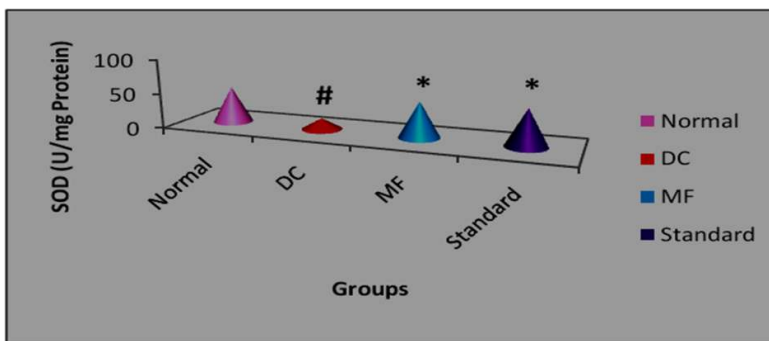
**Fig. 1. Effect of MF on serum insulin levels**

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



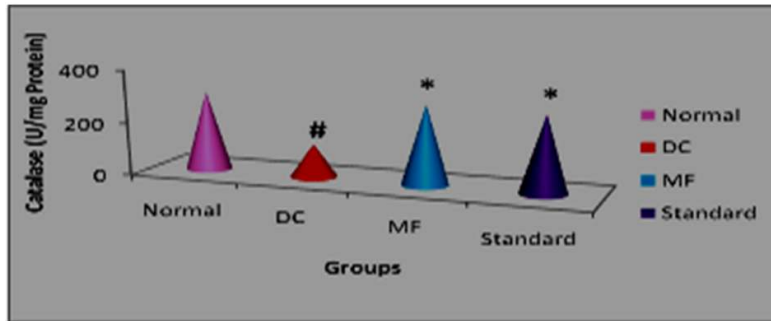
**Fig. 2. Effect of MF on blood Hb and glycosylated haemoglobin (HbA1c) levels**

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



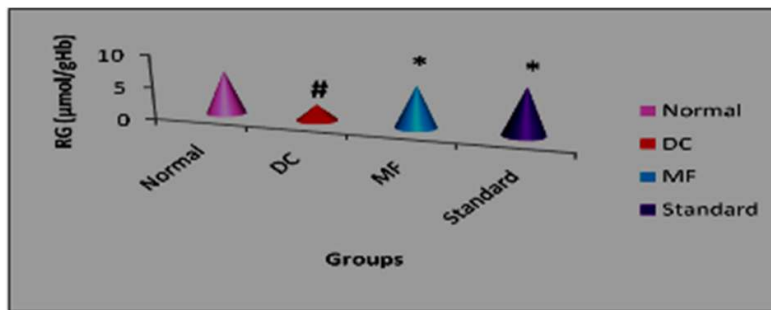
**Fig. 3. Effect of MF on SOD**

Values are expressed as mean  $\pm$  S.E.M, n=6 in each group  
 #p < 0.01 when compared to normal, \*p < 0.01 when compared to diabetic control



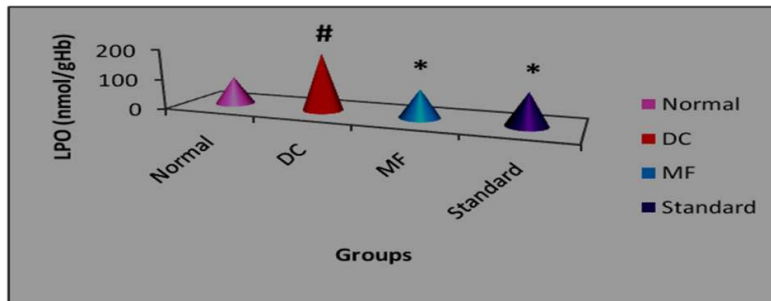
**Fig. 4. Effect of MF on Catalase**

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



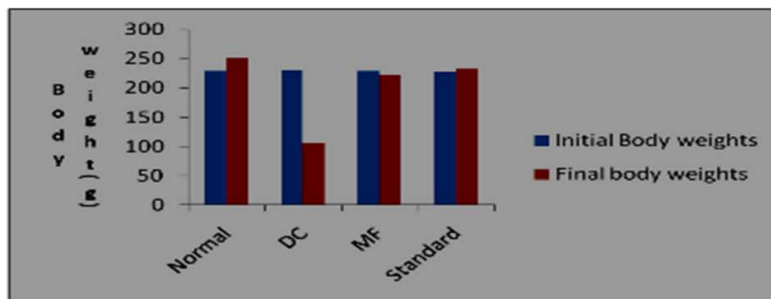
**Fig. 5. Effect of MF on Reduced glutathione**

Values are expressed as mean  $\pm$  S.E.M, n=6 in each group  
 #p < 0.001 when compared to normal, \*p < 0.01 when compared to diabetic control

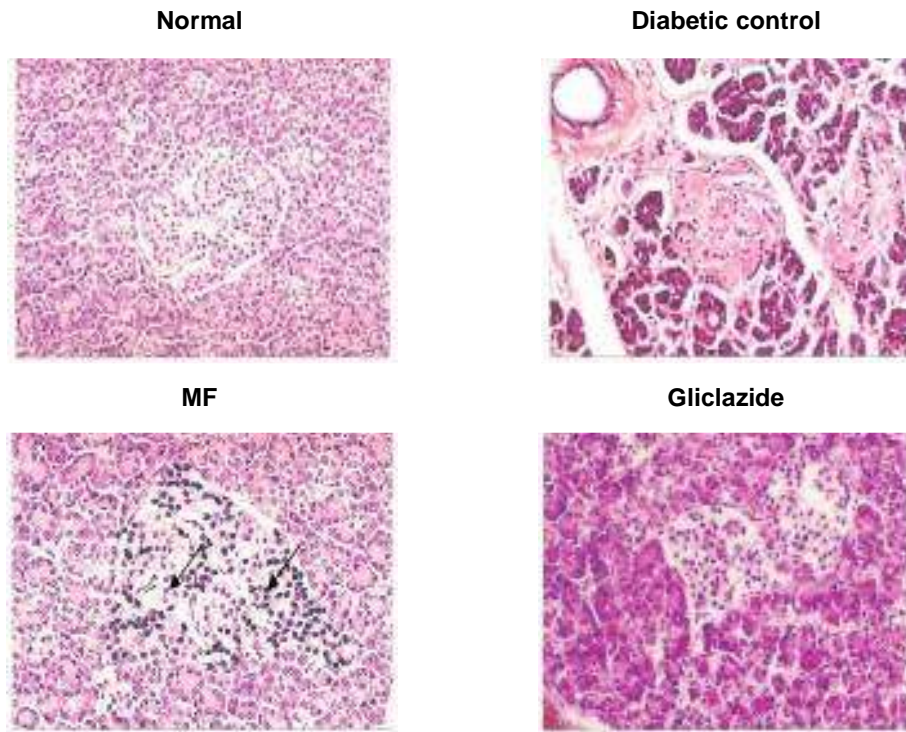


**Fig. 6. Effect of MF on lipid peroxidation**

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



**Fig. 7. Effect of MF on body weight**



**Fig. 8. Histology slides**

In the present study, streptozotocin was chosen to induce experimental diabetes in rats. Streptozotocin induced hyperglycemia has been described as a good experimental model to study diabetes mellitus [21]. Its administration to rats showed an increase in the blood glucose levels and a decrease in the plasma insulin levels. Findings of the present investigation also revealed that STZ induced diabetes resulted in a decrease in Hb levels and elevation in glycosylated haemoglobin (HbA1c) level. Furthermore STZ significantly increased oxidative stress indicated by decrease in blood SOD, Catalase, GSH and increase in serum MDA levels. In the present study, Gliclazide was used as standard because it is an effective oral hypoglycemic agent which reduces blood glucose levels in animal models of diabetes as well as in diabetic patients.

Data of current study showed that different fractionated extracts exhibited antihyperglycemic activity but MF 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 MF 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 blood SOD, catalase, GSH and significant decrease in serum MDA levels were observed in animals treated with MF for 30 days when compared to diabetic control group.

The more significant antidiabetic activity of MF may be due to the presence of flavonoids and saponins. Literature showed that flavonoids and saponins are good antidiabetic metabolites [22]. Other fractions lacked these metabolites and this may account for their non-significant antidiabetic activity. Alkaloids and tannins have similarly been implicated in the antidiabetic activities of plant [23,24].

## 5. CONCLUSION

From the present study it could be concluded that MF decreased STZ induced hyperglycemia and ameliorated oxidative stress. Its antidiabetic activity may be related to insulinomimetic and antioxidant action. Observed antidiabetic and antioxidant activity of the title plant may be attributed to bioactive flavonoids. The present study reports for the first time to our knowledge



that *D. aegyptium* possesses antidiabetic activity in streptozotocin induced diabetic rats. The major outcome of the study is to provide a platform for the application of whole plant of *D. aegyptium* to supplement existing oral antidiabetic drugs and this research also supports the inclusion of this plant in traditional antidiabetic preparations and the formulations made using these identified effective extracts and fraction of this plant could serve the purpose better than the existing formulations with crude extract. We need further study to determine the mechanism of action and to isolate the active principles responsible for antidiabetic activity.

### CONSENT

It is not applicable.

### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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