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Anti-diabetic and anti-hyperlipidemic effects of methanol extracts of *Chloris barbata*(SW.) in Streptozotocin- induced diabetic rats

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ABSTRACT

The leaf of Chloris barbata (SW.) belongs to the family Poaceae is used in Indian system of traditional medicine to treat diabetes mellitus. The present study investigates the in vivo anti-diabetic and anti-hyperlipidemic activities of methanolic extract Chloris barbata (SW.) (MECB) leaves in normal, glucose-loaded hyperglycemic and streptozotocin (STZ) induced diabetic rats. Acute toxicity was studied in rats after the oral dose of MECB to determine the dose to assess the anti-diabetic activity. Oral administration of MECB did not exhibit toxicity and mortality at a dose of 2000mg/kg. MECB (100, 200 and 400 mg/kg) was administered to STZ (40 mg/kg, i.p) induced diabetic rats for 28 days. The three doses of MECB showed a significant decrease in blood glucose and significant increase in plasma insulin and liver glycogen levels in treated diabetic rats. Further, MECB showed anti-hyperlipidemic activity as evidenced by significant decrease in serum TC, TG, LDL-C, VLDL-C levels and significant increase in HDL-C level in treated diabetic rats. MECB also restored the altered plasma enzymes such as SGOT, SGPT and ALP, total protein, liver glycogen levels to near normal. The effect of MECB was comparable to the standard drug glibenclamide. Results of this experimental study indicated that MECB possessed anti-diabetic and anti-hyperlipidemic activities.

Keywords: *Chloris barbata,* Anti-diabetic, Anti-hyperlipidemic, SGOT, SGPT, ALP, Insulin, Liver glycogen.

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder caused by an absolute or relative lack of insulin and or insulin resistance, which results in hyperglycemia and abnormalities in carbohydrate, protein and fat metabolism. Diabetes mellitus is increasingly common metabolic disorder and one of the five leading causes of death in to the world. It is projected that 300 million people will have the disease by the year 2025[1]. Though different types of oral hypoglycemic agents are available along with insulin in the treatment of diabetes mellitus, there is a growing interest in herbal remedies due to the side effects associated with these therapeutic agents[2]. The traditional plant is an

important aspect for the development of new drug formulations, being passed and recent history of drug discovery in exorably bound to plant kingdom[3,4]. Hence traditional medicine experience is precious in identifying possible target particular part of plant species and also suggesting which kind of extraction should be used. The investigation of anti-diabetic drugs of plant origin which are used for traditional medicine is thus of great importance

The plant *Chloris barbata*(SW.) belongs to the family Poaceae, formerly called as Graminae, grass family of the monocotyledonous flowering plants, a division of the order Poales. *Chloris barbata*(SW.) is distributed all over the world, and it is a very common weed of plains, wastelands. In Chittoor district, it is found in cultivated fields, forests and waste lands[5]. The entire plant of *Chloris barbata*(SW.) is used to treat rheumatism in traditional medicine[6]. The leaf paste of *Chloris barbata*(SW.) is used externally for skin disorders leaves juice used in fever, diarrhea and diabetes[7]. Analgesic and anti-inflammatory activities of petroleum ether extract of *Chloris barbata*(SW.) has reported[8]. Traditionally, *Chloris barbata*(SW.) has been used in treatment of diabetes, no scientific report is available and so the present study has been carriedout to investigate the Anti-diabetic and anti-hyperlipidemic activities of methanol extracts of *Chloris barbata*(SW.) in Streptozotocin-induced diabetic rats.

MATERIALS AND METHODS

Plant material

The leaves of *Chloris barbata*(SW.) was collected from the cultivated fields of Chandragiri, Chittoor district of Andhra Pradesh, India in December 2010. The plant was authenticated Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, Sri Venkateswara University, Tirupati.

Preparation of Extract

The leaves of *Chloris barbata*(SW.) was dried in shade and pulverized in the grinder-mixer to obtain a coarse powder, then passed through the 40 mesh sieve. A weighed quantity (85gm) of powder was subjected to continuous hot extraction with methanol in soxhlet apparatus for 48hours. Then the extract was evaporated at reduced pressure using rotary evaporator until all the solvent has been removed to give an extract sample. The percentage yield of methanol extract of *Chloris barbata*(SW.) was found to be 6.5% w/w.

Phytochemical investigation

The phytochemical examination of methanol extract of *Chloris barbata*(SW.) leaves was performed by the standard methods[9].

Animals used

Albino Wistar rats(200-250gm) were obtained from the animal house in Sree Vidyanikethan College of Pharmacy, Tirupati, Andhra Pradesh. The animals were maintained in a well-ventilated room with 12:12 hour light / dark cycle in polypropylene cages and fed with standard pellet feed (Hindustan lever limited, Bangalore) and water was given adlibitum. The animals were maintained under standard housing conditions (room temperature 24–27◦C and humidity(60–65%). The experiments were performed after approval of the protocol by the Institutional Animal Ethics Committee (IAEC) and were carried out in accordance with the current guidelines on the care of laboratory animals.

Acute Toxicity Study

The acute toxicity MECB was determined as per the OECD guideline no. 423(Acute toxic class method). Albino wistar rats(n=6) either sex selected and kept fasting for overnight providing only water. MECB was administered orally at the dose level of 2000mg/kg by oral needle and observed for 14days[10]. The animals were observed for gross behavioral, neurological, autonomic and toxic effects at short intervals of time for 24h and then daily for 14days. It was observed that the MECB was not mortal even at 2000mg/kg doses. Hence, 1/20th(100mg/kg), 1/10th(200mg/kg) and 1/5th(400mg/kg)of the doses were selected for further study[10].

Evaluation of MECB in oral glucose tolerance test

Assessment of oral glucose tolerance test (OGTT) of MECB Overnight fasted normal rats were divided into five groups of six rats each. They were orally administered with vehicle, MECB(100, 200 and 400mg/kg) and glibenclamide(600µg/kg), respectively. Glucose(2g/kg) was fed 30min after the administration of extract and glibenclamide[11]. Blood was withdrawn through the tail vein at 0, 30, 60 and 120 min of glucose administration

and glucose levels were estimated by commercially available glucose strips (Accu-Chek) using one-touch glucometer (Johnson-Johnson, India).

Experimental Induction of non-insulin dependent diabetes mellitus

Diabetes mellitus was induced by single intraperitoneal injection of freshly prepared STZ(40mg/kgb.wt) in 0.1 Mcitrate buffer (pH4.5) in a volume of 1ml/kgb.wt. STZ injected rats were given 20% glucose solution for 24h to prevent initial drug-induced hypoglycemic mortality[12]. Diabetes was confirmed by the elevated glucose levels in plasma, determined at 96h. Rats with a fasting blood glucose level>250mg/dl were considered diabetic and used in the study.

Experimental design

Three-month old male albino Wistar rats weighing 180-250gms were used. The animals were randomly divided into six groups of six animals each.

Group I–Normal untreated rats (1%w/v CMC; 5ml/kg, b.w.p.o)
Group II-diabetic rats received only vehicle (1%CMC; 5ml/kg, b.w.p.o)
Group III–diabetic rats received the MECB (100mg/kg/day p.o) suspended in 1% w/v CMC.
Group IV–diabetic rats received the MECB (200mg/kg/day p.o) suspended in 1% w/v CMC.
Group V-diabetic rats received the MECB (400mg/kg/day p.o) suspended in 1% w/v CMC.
Group VI–diabetic rats received Glibenclamide (600µg/kg p.o) suspended in 1% w/v CMC.

Treatment was given orally using an intragastric tube once daily for 28days, continuously. Blood glucose was estimated on 0th, 7th, 14th, 21th and 28th day by commercially available glucose strips (Accu-Chek) using one-touch glucometer(Johnson-Johnson, India).The initial and final body weights were measured.

Biochemical estimation

On 28th day, the animals were fasted for 12h, anaesthetized and sacrificed by decapitation. Blood samples were withdrawn from cardiac puncture and collected in an eppendorftube. It allowed to coagulate at an ambient temperatures for 30min. Serum was separated by centrifugation at 2000 rpm for 10min for the estimation of plasma insulin, SGOT, SGPT, ALP and total protein[13,14]. Hepatic glycogen level was estimated by the method of Plummer(1978)[15]. Serumtotal cholesterol (TC), triglycerides (TG), LDL-C, VLDL, HDL-C were measured by commercially available diagnostic kits (Span Diagnostics, India). The serum low-density lipoprotein(LDL) and very low-density lipoprotein(VLDL) levels were calculated by Friedewald formula: VLDL=TG/5; LDL=TC-(HDL+VLDL).

Histopathological studies on a pancreas

The freshly removed pancreas was washed with saline and preserve din10% formaldehyde solution for histopathological studies. Paraffin sections of 5µm were obtained from a rotational micro to me and stained with haematoxylin and eosin. The microscopic slides were photographed. The injuries of the pancreas were assessed by histopathological changes as follows[16]:

Normal-The normal numbers and volume of islet cells;
Minor injury – The numbers of islet cells were slightly lower and slightly swollen;
Moderate injury – The numbers of islet cells were moderately lower and moderately swollen;
Obvious injury – The numbers of islet cells were obviously lower and obviously swollen;
Severe injury – The numbers of islet cells were severely reduced and severely swollen.

Statistical analysis

The data were expressed as mean ± standard error mean (S.E.M). The Significance of differences between the groups was assessed using one way and multiple way analysis of variance (ANOVA). The test followed by Dunnett's test p values less than 0.05 were considered as significance.

RESULTS

Phytochemical investigation

The results of preliminary phytochemical investigation of the methanol extract of *Chloris barbata*(SW.) leaves shows the presence of flavonoids, tannins, saponins and proteins.

Acute toxicity study

The acute toxicity studies revealed that the oral administration of methanol extract of *Chloris barbata*(SW.) leaves at a dose of 2000 mg/kg, body weight. Did not cause any mortality and considered as safe. No adverse effect on the general behavior or appearance of the rats and all the rats survived during the whole experimental period.

Effects of methanol extract of *Chloris barbata*(SW.) leaves(MECB)on oral glucose tolerance tests

After glucose load (2g/kg), the administration of MECB at the doses of 100, 200 and 400 mg/kg decreased the elevation of serum glucose level significantly ($p<0.01$) at 30 and 60 min. Table1 shows the oral glucose tolerance of MECB.

Effects of methanol extract of *Chloris barbata*(SW.) leaves(MECB)on the Plasma glucose level of STZ induced diabetic rats

Plasma glucose level was measured in normal and experimental rats on day 0, 7, 14, 21 and 28 of drug treatment. STZ-induced diabetic rats showed a significant increase in the levels of blood glucose when compared to normal rats. After treatment with MECB at 100, 200 and 400mg/kg the blood glucose was significantly($P<0.01$) reduced compared to the first day. The standard drug glibenclamide treated rats also showed significant ($P<0.01$) reduction in plasma glucose level(Table2).

Histopathological studies on the pancreas

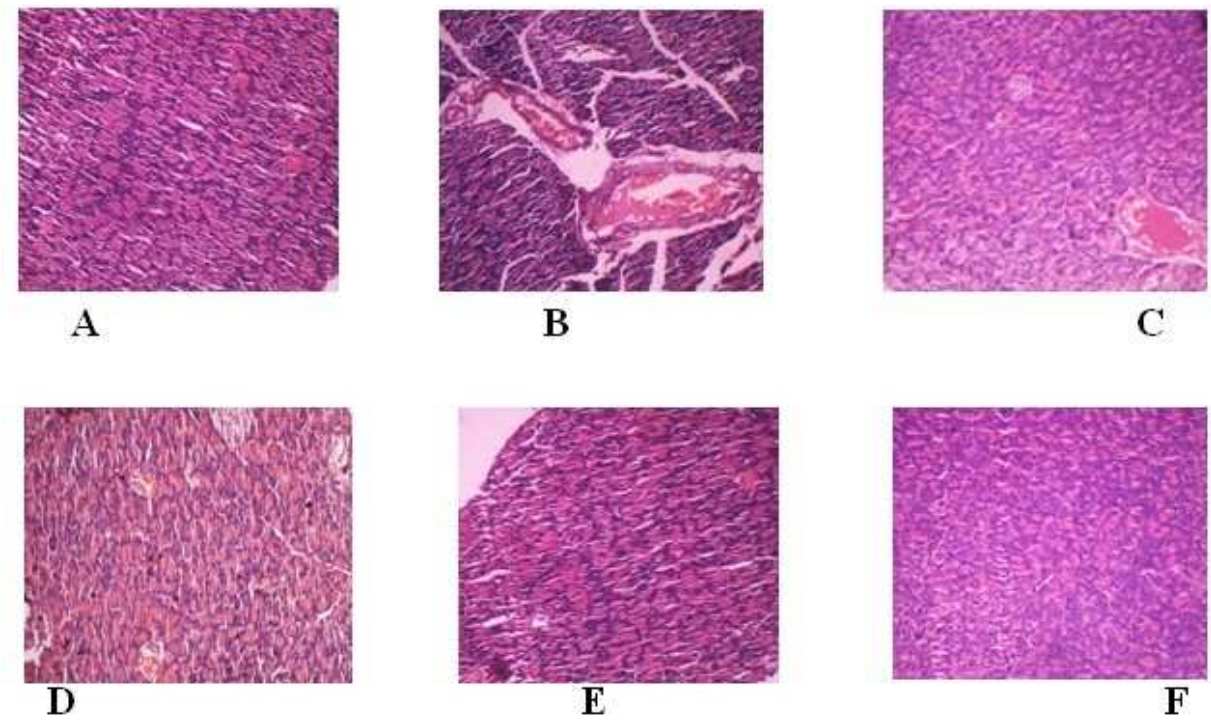

Fig. 1. (A) **Normal control**; islets with normal cellular characteristics (B) **Diabetic control**; Vascular degranulatedislets with severely reduces the volume and number of islets (C) Diabetic rats treated with **MECB100mg/kg**; mild granulationislets with moderately reduced volume and number (D) Diabetic rats treated with **MECB200mg/kg**; moderate granulationislets with slightly reduced volume and number. (E) Diabetic rats treated with **MECB400mg/kg**; well granulatedislets with normal cellular characteristics. (F) Diabetic rats treated with **Glibenclamide600µg/kg**; granulated islets with normal cellular characteristics.

Table 1. Effects of MECB on oral glucose tolerance
*All values compared with normal control groups. * p < 0.05 ; ** p < 0.001*

Groups	Serum Blood Glucose (mg/dl)			
	0min	30min	60min	120min
Normal control	95.33±1.202	125.83±0.6009	117±0.7303	102.67±0.6146
MECB (100 mg/kg/day p.o)	97.17±1.4	121.5±0.8466*	113±0.8165**	100.83±0.7923
MECB (200 mg/kg/day p.o)	95±1.844	119.67±0.7149**	107.83±0.7032**	98.17±1.014**
MECB (400 mg/kg/day p.o)	94.5±1.204	118.5±0.4282**	101.83±0.7032**	92.17±0.5426**
Glibenclamide (600 µg/kg p.o)	96±2.017	97.33±2.060**	97.5±0.6708**	85.5±0.8466**

Table 2. Effects of MECBon Serum Blood Glucose in STZ induced diabetic rats
*All values compared with diabetic control groups. * p < 0.05 ; ** p < 0.01*

Groups	Serum Blood Glucose (mg/dl)				
	day 0	day 7	day 14	day 21	day 28
Normal control(1% w/v CMC)	105.17±1.138	106±1.125	102.33±1.667	99.83±0.8724	98.33±0.8819
STZ induced Diabetic control	316.5±2.566	320.67±2.186	315.67±2.06	310.17±2.676	314.5±1.746
STZ+ MECB (100 mg/kg)	310.5±2.861	296.33±1.43	264.83±2.04*	194.17±2.088**	148.67±2.333**
STZ+ MECB (200 mg/kg)	311.83±1.493	273.17±1.905*	244.17±1.40**	181.17±2.212**	127.83±2.056**
STZ+ MECB (400 mg/kg)	311.67±2.716	262.67±1.783*	219.33±1.542**	168±1.949**	113.67±2.996**
STZ+ Glibenclamide (600 µg/kg)	311.5±3.766	259±1.862*	213.17±1.740**	154±1.949**	96±2.696**

Biochemical estimation

Effects of MECB on serum transaminase, total protein, plasma insulin and liver glycogen

Table3represented that the efficacy of MECB on serum SGOT, SGPT, ALP, total protein, plasma insulin and liver glycogen in diabetic rats. In diabetic rats, administration of MECB at the doses of 100, 200 & 400mg/kg and glibenclamide significantly (P<0.01) reduced the serum SGOT, SGPT, ALP and significantly (P<0.01) increases the total protein, plasma insulin, liver glycogen towards normal level when compared to diabetic control rats. The MECB 200 & 400mg/kg significantly (P<0.01) higher reduction of serum SGOT, SGPT, ALP and increases the total protein, plasma insulin and liver glycogen towards normal level.

Effects of MECB on body weight

The body weight of diabetic control rats significantly decreased (P<0.01) when compared to normal control rats. MECB at100, 200 & 400mg/kg and glibenclamide treated rats showed slight weight gain on day 28 relative to day 0 when compared to diabetic control rats as shown in table 3.

Effects of MECB on lipid profile

In diabetic rats, Serum TC, TG, LDL-C, VLDL-C levels were increased and HDL-C levels were significantly (P<0.05) decreased when compared to normal control rats. In diabetic rats, administration of MECB at the doses of 100, 200 & 400mg/kg and glibenclamide significant (P<0.05) reduction in elevated SerumTC, TG, LDL-C, VLDL-C and significantly (P<0.01) increases the HDL-C levels when compared to diabetic control rats (Table4).

Histopathological studies on the pancreas

Histopathological analyses revealed that administration of STZ caused severe injury to the pancreas, such as a decrease as the number of islet cells and a reduction in the diameter of pancreatic islets. After treatment with MECB 400mg/kg and standard drug glibenclamide showed normal numbers and volume of islet cells. The numbers of islet cells were slightly lower and slightly swollen with MECB100& 200mg/kg.

Table 3. Effects of MECBon SGOT, SGPT, ALP, Liver glycogen, plasma insulin, total protein and changes in body weight in STZ induced diabetic rats

*All values compared with diabetic control groups. * p < 0.05 ; ** p < 0.01*

Groups	SGOT (IU/dl)	SGPT (IU/dl)	ALP (IU/dl)	Liver glycogen(mg/g of tissue)	Plasma Insulin (µU/ml)	Total Protein	Body weight (g)	
							Initial	Final
Normal control (1% w/v CMC)	47.67±1.476**	62.5±1.232**	120.83±1.641**	51.83±1.515**	14.79±0.3633**	7.87±0.0803**	229.5±1.478	240.5±2.446**
STZ induced Diabetic control	121.5±2.045	152±1.77	238±1.693	16.33±0.667	8.08±0.1741	6.17±0.0667	226.33±2.765	193.67±2.728
STZ + MECB (100 mg/kg)	68.33±1.476**	98±2.145**	167.17±1.887**	25±1.00**	11.35±0.959**	6.83±0.076**	223.67±2.94	236.5±2.320**
STZ + MECB (200 mg/kg)	61.17±1.046**	90.67±1.542**	146.67±1.909**	30.33±1.085**	11.97±0.2214**	7.2±0.0557**	223.5±3.99	233.17±3.772**
STZ + MECB (400 mg/kg)	54.17±1.249**	83.17±1.721**	134±1.291**	34.83±1.195**	12.51±0.1316**	7.43±0.0558**	223.17±3.962	235.5±2.094**
STZ + Glibenclamide (600 µg/kg)	53±1.549**	73.5±1.147**	129.33±1.358**	42.17±0.946**	13.15±0.2009**	7.6±0.07303**	224.67±3.981	233±1.549**

Table 4. Effects of MECBon TC, TG, LDL-C, VLDL-C and HDL-C in STZ induced diabetic rats

*All values compared with diabetic control groups. * p < 0.05 ; ** p < 0.01*

Groups	Serum Lipid Profile (mg/dl)				
	Total Cholesterol (mg/dl)	Triglycerides (mg/dl)	LDL- C (mg/dl)	VLDL- C (mg/dl)	HDL- C (mg/dl)
Normal control (1% w/v CMC)	108±1.571**	111.83±2.120**	36.13±1.170**	22.37±0.424**	49.5±1.142**
STZ induced Diabetic control	251.83±2.182	159.67±1.430	188.07±2.485	31.93±0.286	31.83±1.327
STZ + MECB (100 mg/kg)	136.17±1.138**	128.33±2.171**	68.83±1.006**	25.67±0.434**	41.67±0.7601**
STZ + MECB (200 mg/kg)	126.5±2.125**	131.17±1.721**	56.27±2.263**	26.23±0.3442**	44±0.6325**
STZ + MECB (400 mg/kg)	116.67±1.626**	124±1.807**	47.2±1.661**	24.8±0.3615**	44.67±0.9545**
STZ + Glibenclamide (600 µg/kg)	110.17±1.424**	114.67±2.472**	37.73±2.124**	22.93±0.4944**	49.5±1.285**

DISCUSSION AND CONCLUSION

The currently available drug therapies for management of diabetes mellitus have certain drawbacks and therefore there is a need to find safer and more effective anti-diabetic drugs [17]. The aim of the present study was to evaluate the anti-diabetic efficacy of methanol extract of *Chloris barbata* (SW.) leaves in STZ-induced diabetic rats. The experimental diabetic model used in this study corresponds to type 2 diabetes with STZ at dose of 40 mg/kg body weight exerts partial β-cytotoxic effect (DNA strand breakage) with residual β cells secreting insufficient insulin leading to hypoinsulinemia which as a result decreased glucose uptake and hyperglycemia which is the characteristic feature of type 2 diabetes[18, 19].

In the present study, diabetic rats showed significant decrease in plasma glucose level after treatment with MECB, which was similar to glibenclamide. Glucose lowering effect of MECB might be due to stimulation of surviving β-cells of islets of Langerhans leading to of the increases pancreatic secretion of insulin from regenerated β-cells, or its action to release bound insulin from regenerated β-cells by inhibiting ATP sensitive K+ channels like glibenclamide. MECB contained phenolic compounds such as flavonoids and tannins; previous studies showed that phenolic compounds acted on ATP sensitive K+ channels and regulated blood glucose [20].

The marker enzymes like serum SGOT, SGPT and ALP which are considered to be responsible for the cell damage, are reduced by the MECB and therefore it may be presumed that the MECB protects the cellular damage. Glycogen is the primary intracellular storable form of glucose and its level in various tissues is a direct reflection of insulin activity as insulin promotes intracellular glycogen deposition by stimulating glycogen synthase and inhibiting glycogen phosphorylase. The liver glycogen content was markedly reduced in diabetic animals, which is in proportion to insulin deficiency [21]. The glycogen content in the liver increases in the MECB treated group compared to the diabetic rats which could be due to increased insulin secretion[22].

Diabetic rats showed significant reduction in body weights when compared to normal and treated rats, which could be due to poor glycemic control. The excessive protein catabolism is the breakdown of protein in to amino acids for gluconeogenesis during insulin-deprived state results in muscle wasting and weight loss in diabetic untreated rats [23]. The findings of increased protein breakdown (increased leucine flux) and increased amino acid oxidation during insulin deficiency are consistent with the observed anti-catabolic effect of insulin on protein metabolism. Rise in insulin levels upon treatment with MECB in diabetic rats resulted in improved glycemic control, which prevented the loss of body weight.

The secondary complications of diabetes are atherosclerosis and coronary heart disease which developed by primary factors of hypercholesteremia and hypertriglyceridemia [24-26]. MECB significantly reduced Serum TC, TG, LDL-C, VLDL-C in STZ-diabetic rats while the MECB treated groups showed increased value of HDL-C. Thus, it is reasonable to conclude that MECB could modulate blood lipid abnormalities. The hypolipidemic effect of MECB could be activation of lipoprotein lipase and hydrolyzes the triglycerides by metabolism of insulin [27-29]. The repeated administration of MECB for a period of 28 days resulted in a significant improvement in lipid parameter levels when compared to diabetic control rats.

Histopathological analyses revealed that oral treatment with MECB has protect and regenerate the islets by increases the number and diameter of pancreatic islets, which suggests that the anti-diabetic activity of the MECB was due to an extra-pancreatic effect and independent of insulin secretion. The administration of STZ causes severe damage to the pancreas, such as a reduction in the number and diameter of pancreatic islets, which was not reversed by the MECB.

The present study results concluded that the administration of the methanol extract of *Chloris barbata* (SW.) leaves has the ability to reduce glucose metabolism disorders in type 2 diabetes, thereby confirming its ethnomedicinal use. Thus, the significant anti-diabetic and anti-hyperlipidemic activity of MECB could be due to the presence of various phytoconstituents found in the phytochemical screening which alone or in synergism can impart therapeutic effect. However, the study of the active principles present in the leaves of *Chloris barbata* (SW.), particularly of the flavonoids, may represent an interesting alternative in the treatment of diabetes.

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