The protective effect of *Matricaria pubescens* extracts against alloxan-induced hyperglycemia in rats

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Abstract

The purpose of this research is to look into the potential inhibitory capacity of *Matricaria pubescens* methanolic extract (EMMP) on key diabetes-related enzymes in diabetic rats. A significant increase in serum and pancreatic α-amylase activity in untreated diabetic rats (Diab) of 61% and 75%, respectively, resulted in an 184% increase in blood glucose levels compared to controls. However, compared to untreated diabetic rats, treatment with the extract (Diab + EMMP) resulted in a significant decrease in pancreatic α-amylase activity in the pancreas (15%) and serum (28%). As a result, a significant reduction in blood sugar level has been observed, reaching 28%. When rats treated with EMMP were compared to untreated diabetics, the level of glycated hemoglobin (HbA1c) decreased by 28%. Furthermore, a significant increase in pancreatic serum lipase activity (59%) in untreated diabetic rats treated with EMMP resulted in a significant inhibition (21%). In diabetic rats treated with EMMP, β-cells were found to have a powerful protective effect. However, we observed body weight recovery in diabetic rats treated with EMMP.

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Introduction

Herbal medicines have recently gained prominence in primary care needs; they are the foundation of new medical products and their derivatives.[1] Natural plant-based antioxidants, whether in the form of raw extract or bioactive compounds, are extremely effective at preventing oxidative stress, which causes cell damage.[2]

Diabetes Mellitus (DM) is a metabolic disorder characterized by a variety of symptoms that lead to insulin deficiency,[3] as well as chronic hyperglycemia and changes in fat, protein, and carbohydrate metabolism. According to the World Health Organization, there were approximately 171 million cases of diabetes in 2000, with a projected increase to 366 million by 2030.[4] The current treatment methods have a variety of side effects that impact the patient's quality of life. As a result, herbal therapy is gaining popularity.[5]

In this regard, complementary and alternative medicine may be an effective solution that is less costly and less harmful to the patient. *Matricaria pubescens* is common in southern Algeria's deserts and salt marshes. The organs of this plant are used as an active drug in traditional Algerian medicine to treat rheumatism, gout, and asthma.[6] This study sought to investigate the possible inhibitory power of *Matricaria pubescens* methanolic extract (EMMP) on key diabetes-related enzymes in diabetic rats.

Materials and Methods

Extraction procedure

Two solid-liquid extractions were carried out using a solvent of increasing polarity: methanol (MeOH). Forty grams of powder from the plant (*Matricaria pubescens*) were placed in 100 mL of

solvent. The whole was homogenized and stirred for 48 hours at room temperature. The extracts obtained were filtered and concentrated using a rotary evaporator.

Biological material (animals)

The study was performed in male rats of the Wistar strain weighing 140 ± 23 g. They were placed in an animal facility with a temperature of 22 ± 2 °C with an alternation of 12 hours of light and 12 hours of darkness, the relative humidity being around 40%. Throughout the experimentation period, the animals were treated according to the ethical rules inherent in animal experimentation (approval number: FST/LNFP/Pro 152012).

Toxicity test

A dose-effect test was performed for the methanolic extract of the seeds of *Matricaria pubescens* at 10, 20 and 50 mg/kg of Body Weight (BW). As a result, we chose 50 mg/kg of BW as the most effective of the three tested doses to administer to diabetic rats. Furthermore, the toxicity of our extracts has been tested and found to be non-toxic up to 5 g/kg BW. The treated rats were given daily doses of the corresponding treatment by gavage at a predetermined time of day for 30 days. Furthermore, the weight change of the rats in the different batches was monitored throughout the experiment, both before and after the induction of alloxan diabetes and during treatment.

Pancreatic α-amylase activity assay

The measurement of α-amylase activity is carried out with the 2-chloro-4-nitrophenyl-α-D-maltotrioside (CNPG3) method using a kit from Biolabo, France (Ref. 99523). It is the most efficient method in terms of linearity, sensitivity and accuracy.

Blood glucose determination

The glycemia was determined by the enzymatic glucose oxidase method using a kit from Biolabo, France (Ref. 80009).

Glycated hemoglobin (HbA1c) assay

The HbA1c assay was carried out using a kit from Biolabo, France (Ref. 22010) using a turbidimetric measurement comprising the successive addition of two reagents; the first reagent (R1) contains anti-HbA1c antibodies, which form soluble complexes with HbA1c; the second reagent (R2) contains polyhaptens capable of binding to the antibodies which have remained free, in the form of insoluble complexes. The measured signal is inversely proportional to the HbA1c level of the sample and is compared with that of a five-point calibration curve (measurement range: 2 to 24.8 g/L of HbA1c). The HbA1c level is calculated relative to the total hemoglobin concentration, determined in the same sample after lysis of the red blood cells and spectrophotometric measurement of the cyanmethemoglobin.

Determination of Total Cholesterol (Ch-T), triglycerides, High-density lipoproteins (HDL)-cholesterol and Low-density lipoproteins (LDL)-cholesterol

The cholesterol assay was carried out by the enzymatic cholesterol oxidase method using a kit from Biolabo, France (Ref. 80106). The determination of triglycerides was carried out by the enzymatic glycerol kinase method using a kit from Biolabo, France (Ref. 80019). The HDL-Cholesterol assay was performed by an enzymatic method using a Kit from Biolabo, France (Ref. 90206). The serum LDL-Cholesterol level is determined by simple calculation based on the formula of Friedewald.[7]

Pancreatic lipase activity assay

The determination of the activity of pancreatic lipase in the serum was carried out by an enzymatic method using a kit from Biolabo, France (Ref. 99891).

Pancreas sampling

Pancreas samples were taken from the different groups of male rats. Some of them were immediately placed in a fixative for subsequent histological sections, others were ground in phosphate buffer (0.1M; pH = 7.6) and then centrifuged at 5000 × g, 20 min and 4 °C to perform the assay of certain biochemical parameters. To determine the concentration of TNF-α in pancreatic tissue, 100 mg of each pancreas used in this test were ground in phosphate buffer (pH = 7.2). The ground material was then centrifuged at 2000 × g for 10 minutes and the collected supernatant was stored at -20 ° C.

Diabetes pathology

Our study on the antidiabetic activity of *Matricaria pubescens* was carried out in male Wistar rats. The condition of experimental diabetes was induced by alloxan.

In order to obtain diabetic rats, the rats were first fasted for at least 12 hours. Then an alloxan monohydrate solution (Sigma, St. Louis, Mo, USA) was prepared by dissolving the product in saline solution (pH 4.5). A single dose of 150 mg/kg BW was injected intraperitoneally into each rat.[8] After 6h, each diabetic rat received orally 5 to 10 mL of a glucose solution (20%). During the next 24 h, a glucose solution (5%) was supplied *ad libitum* to the treated rats. One week after the injection of alloxan, blood glucose was measured using a glucometer (Bionime, Switzerland) by section of the end of the tail of each rat. The rats which presented a glycemia higher than 2 g/L were considered diabetic and included in this study.[9]

Adult male rats were divided into four groups depending on the treatment they received. Each group (8 rats) received standard food and *ad libitum* water. Four distinct groups were considered: a group of control rats (Control); one group (EMMP) received by gavage a *Matricaria pubescens* extract solution at a rate of 50 mg/kg BW, for 8 days; a group of rats made diabetic by alloxan at a rate of 150 mg/kg BW (Diab); a group of diabetic rats which received the extract at a rate of 50 mg/kg BW by gavage (Diab + EMMP) for 30 days. The rats of the groups (controls and untreated diabetics) received a physiological water solution under the same conditions by gavage.

Histological study

During this study, we used the technique described by Gabe.[10]

Statistical analysis

Data were expressed as mean ± standard error of mean (SEM). Statistical analysis was performed with XLSTAT 2012. Duncan's Multiple Interval Analysis of Variance was used to examine the difference between groups. Statistical significance was accepted at the $p<0.05$ values.

Results

Clinical observations in diabetic rats

Alloxan intraperitoneal injection in male rats resulted in 5% mortality in the entire injected population. However, no deaths

were observed in diabetic rats treated with *Matricaria pubescens* extract or in other groups of diabetic and control rats throughout the treatment period. The other experimental groups showed no difference in behavior, outward appearance, or motility when compared to the control group.

Track changes in the animals body weight

The body weight of the animals was monitored throughout the 30 days of our study: before the alloxan injection and after the EMMP treatment period. Table 1 shows the change in mean body weight of the different groups of control, untreated diabetic, and diabetic rats treated with EMMP. Diabetes resulted in a 31% decrease in body weight gain in untreated diabetic rats compared to control animals ($p<0.05$). In contrast to the diabetic rats that survived, we observed body weight recovery in the diabetic rats treated with EMMP.

The effect of EMMP on α-amylase activity and blood sugar level

The Table 2 revealed a significant increase in pancreatic and the serum α-amylase activity of untreated diabetic rats (Diab) by 61% and 75%, respectively, which produced a remarkable rise in blood glucose levels of 184% compared with the controls ($p<0.01$). However, the treatment of diabetic rats with the extract (Diab + EMMP) caused a significant decrease in pancreatic α-amylase activity in the pancreas (15%) and in serum (28%) compared to untreated diabetic rats ($p<0.05$). Therefore, a considerable reduction in blood sugar level has been observed reaching 28%.

For evaluating the effect of EMMP oral administration on glucose homeostasis and to confirm its inhibitory action on digestive carbohydrate enzymes, we performed Oral Glucose Tolerance Test (OGTT) in fasting and conscious rats. The results clearly show that the EMMP oral administration significantly reduced the peak glucose concentration just 60 minutes after glucose administration, compared to untreated diabetic rats (Figure 1).

The effect of oral treatment with EMMP on glycated hemoglobin

Figure 2 represents a significant increase in the level of HbA1c in untreated diabetic rats (+ 95%) compared to normal rats ($p<0.05$). While, a significant decrease in the HbA1c level (28%) was observed in rats treated with EMMP compared to diabetics untreated.[11]

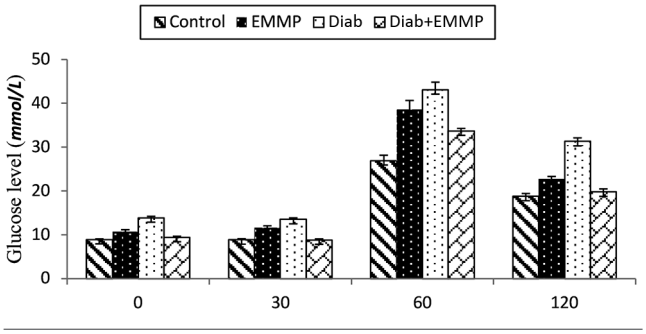

Figure 1. Antihyperglycemic activity of *Matricaria pubescens* methanolic extract (EMMP); untreated diabetic rats (Diab). Values are expressed as mean±SD.

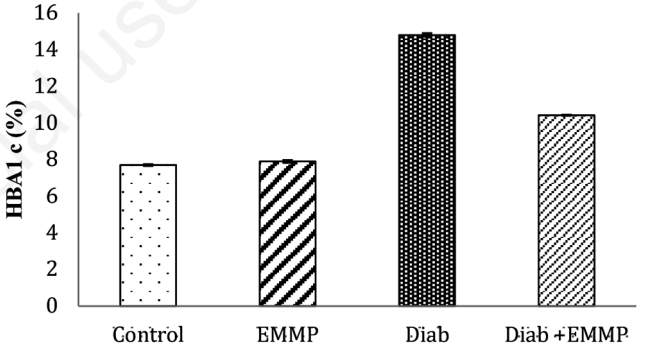

Figure 2. Change in glycated hemoglobin (HbA1c) level in control animals, untreated diabetics (Diab) and diabetics treated before and after the injection of alloxan and after the treatments (Diab+EMMP). EMMP: *Matricaria pubescens* methanolic extract.

Table 1. Evolution of body weight of the control animals, untreated diabetics and diabetics treated before and after the alloxan injection and after treatments.

Parameters	Control	EMMP	Diab	Diab+EMMP
Initial body weight (g)	132.4±2.46	134.58±1.34	133.24±1.13	131.57±2.08
Final body weight (g)	154.4±1.66	152.2±0.7	139.1±0.98	144.2±1.55
Weight gain (g)	22.3±1.02	18.4±1.7	7.1±0.72*	13.3±1.46#

Values are statistically presented as follows: *$p<0.05$ significant difference from controls; #$p<0.05$ significant difference compared to untreated diabetic rats (Diab). EMMP: *Matricaria pubescens* methanolic extract.

Table 2. Evolution of α-amylase activity and blood sugar levels in control animals, untreated diabetics and diabetics treated before and after the injection of alloxan and after the treatments.

Parameters	Control	EMMP	Diab	Diab + EMMP
Pancreatic α-amylase (UI/L)	404.7±5.71	411.4±5.38	652.8±3.79*	552.1±2. 8#
Plasma α-amylase (UI/L)	172.5±4.75	164.1 ±2.13	301.4±1.51*	216.9±5.93#
Blood sugar level (mg/dL)	8.81±0.21	8.59±0.08	25.28±0.7*	18.34±0.70#

Values are statistically presented as follows: *$p<0.05$ significant difference from controls; #$p<0.05$ significant difference compared to untreated diabetic rats (Diab). EMMP: *Matricaria pubescens* methanolic extract.

Histopathology of the pancreas

Figure 3 depicts a light microscope examination of a rat pancreas section. The control and EMMP groups showed normal pancreatic structure of islets of Langerhans and acinus. The pancreas of untreated diabetic rats, on the other hand, showed β-cell degeneration and islet atrophy. In diabetic rats treated with EMMP, however, a potent β-cell protective action was observed.

Effect of EMMP treatment on lipase activity, lipid profile and total protein level

Usually, diabetes mellitus is associated with hyperlipidemia. For this reason, the enzymatic activity of pancreatic lipase in serum has been determined as it is the enzyme responsible for digestion and absorption of lipids in the gastrointestinal tract. In addition, four lipid parameters were evaluated, Total Cholesterol (Ch-T), Triglycerides (TG), High Density Lipoprotein cholesterol (HDL-c) and Low Density Lipoprotein cholesterol (LDL-c) (Figure 4). In untreated diabetic rats, a significant increase in serum pancreatic lipase activity of 59% ($p < 0.05$) was shown compared to control rats. However, treatment of diabetic rats with EMMP resulted in a remarkable inhibition of pancreatic lipase activity in serum (21%) compared to untreated diabetic rats.

Disruption of pancreatic lipase activity affected lipid balance, significantly increased serum Ch-T, TG, LDL-c levels (59%, 51%, and 103%, respectively) associated with a significant decreased HDL-c level (62%) in untreated diabetic rats compared to controls (Table 3). However, the administration of EMMP caused a significant decrease in serum Ch-T, TG, LDL-c concentrations in diabetic rats by 33%, 19% and 41%, respectively. This is associated with a considerable increase in serum HDL-c concentration (+14%) compared to untreated diabetics. In addition, the concentration of total proteins was significantly reduced by 20% in untreated diabetic rats compared to normal rats. While the treatment of diabetics with EMMP induced a restoration of total protein levels compared to untreated diabetics.

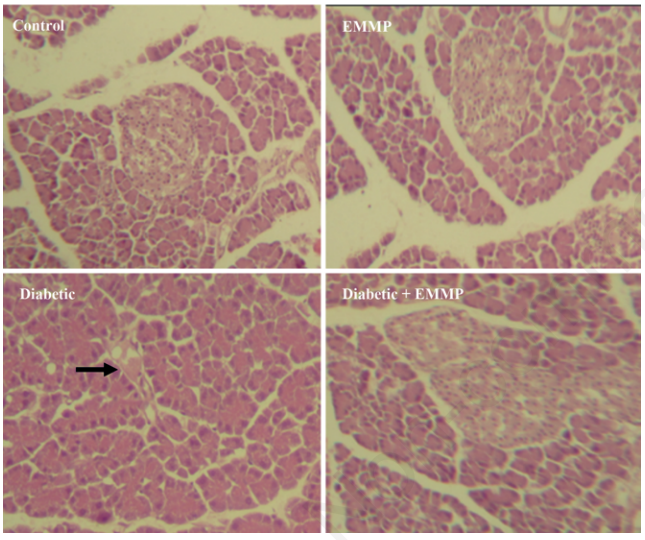

Figure 3. Histology of the pancreas (stained with Hematoxylin & Eosin) of normal rats (Control), normal rats treated with *Matricaria pubescens* methanolic extract (EMMP), untreated diabetics (Diab), and diabetics treated with EMMP (Diab + EMMP). ➔ β-cell necrosis.

Discussion

Many traditional remedies have been used to control hyperglycemia and associated illnesses, among which herbal remedies occupy an important place. Therefore, there has been increasing interest in the use of herbal phenolic compounds, due to their convenience and ease of application in many pharmacological and cosmetic fields.[12]

The results showed a significant decrease in body weight of untreated diabetic rats compared to normal rats. In fact, alloxan-induced diabetes is characterized by severe weight loss.[13,14] This loss can be attributed to decreased protein anabolism and increased breakdown of structural proteins (muscle wasting) due to the unavailability of carbohydrates as an energy source.[15,16] However, the administration of EMMP improves the body weight of diabetic

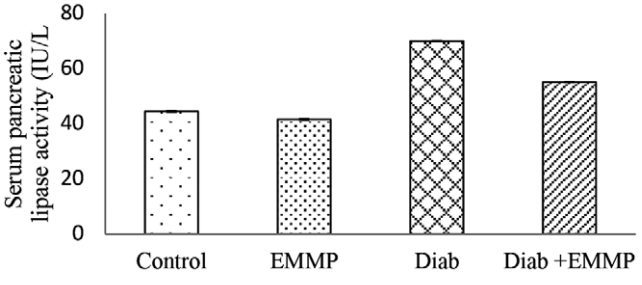

Figure 4. Effect of *Matricaria pubescens* methanolic extract (EMMP) administration on pancreatic lipase activity in serum from different groups.

Table 3. Total Protein Levels and Total Cholesterol (Ch-T), LDL-Cholesterol (LDL-c), HDL-Cholesterol (HDL-c) and Triglyceride (TG) levels in serum and liver of diabetic rats treated with 50 mg / kg BW of *Matricaria pubescens* methanolic extract (EMMP).

Groups	control	EMMP	Diab	Diab+EMMP
Ch-T (mg/dL)	1.34±0.03	1.36±0.05	2.14±0.1*	1.6±0.08#
TG (mg/dL)	1.4±0.1	1.31±0.1	2.21±0.04*	1.78±0.02#
LDL-c (mg/dL)	1.01±0.13	0.78±0.09	2.24±0.08*	1.31±0.05#
HDL-c (mg/dL)	1.27±0.04	1.15±0.05	0.47±0.01*	0.75±0.06#
Total Protein (g/L)	68.46±1.11	67.74±1.02	54.16±2.11*	62.13±1.03#

Values are statistically presented as follows: *$p<0.05$ significant difference from controls; #$p<0.05$ significant difference compared to untreated diabetic rats (Diab), Diabetic Rats treated with EMMP (Diab+EMMP).

animals, compared to that of untreated diabetic rats, thus showing a preventive and protective effect of this extract against the degradation of structural proteins.

The findings also revealed that diabetic rats had a significant increase in serum and pancreatic α-amylase activities, resulting in a significant increase in blood glucose levels. This finding is consistent with previous research.[17] Interruption of glucose uptake through inhibition of α-amylase activity, particularly in the pancreas, can reduce the postprandial increase in blood glucose and may be an important strategy for controlling blood glucose levels in diabetes mellitus.[8,14,18]

In this context, the current study found that giving EMMP to diabetic rats significantly reduced serum and pancreatic levels of α-amylase, which inhibited carbohydrate hydrolysis and absorption and resulted in postprandial blood sugar normalization. The presence of phenolic compounds in the methanolic extract of *Matricaria pubescens* could explain the observed antihyperglycemic effect.[17,19]

The hypoglycemic effect of gallic acid, the main compound identified in the plant studied, has previously been shown to partially alleviate diabetes symptoms in streptozotocin-induced diabetic rats.[20] In contrast, Kamrani *et al.*[21] demonstrated that this phenolic compound inhibits α-amylase activity *in vitro*. This supports our findings that EMMP has an anti-diabetic effect by inhibiting α-amylase activity in the gastrointestinal tract of diabetic rats.

EMMP's hypoglycemic property, on the other hand, could be attributed to the presence of rutin, a powerful bioactive compound capable of increasing glucose uptake by muscle cells[22] or inhibiting the activity of key enzymes in glucose metabolism.[19] At the same time, our histopathological examination of diabetic rats' pancreatic sections revealed an increase in the number of β-cells after EMMP treatment, indicating an improvement in postprandial blood sugar, which was previously confirmed by a glucose tolerance test in diabetic rats. This could lend credence to the notion that various phenolic compounds correct hyperglycemia and produce an insulinogenic effect by regenerating β-cells.

Furthermore, diabetic rats had a significant increase in HbA1c levels when compared to controls. The concentration of HbA1c is thought to be a good diagnostic and prognostic indicator of diabetes complications.[23] When compared to untreated diabetic animals, EMMP treatment reduced HbA1c and increased hemoglobin concentration in diabetic rats. This reflects EMMP's long-term effectiveness in controlling diabetes and its complications.[24,25]

Lipids are important in the pathology of diabetes mellitus. In fact, persistent pancreatic inflammation during experimental diabetes leads to increased pancreatic lipase activity.[14] This is linked to an increase in serum T-Ch, TG, and LDL-c levels, as well as a decrease in HDL-c levels.[26] The findings corroborate these findings and show that giving EMMP to diabetic rats reduced pancreatic lipase activity and improved the lipid profile of the treated animals. Histopathological examination of the pancreas of diabetic rats showed damage to the islets of Langerhans as evidenced by degeneration of β cells. However, partial regeneration of islets of Langerhans was observed in diabetic rats treated with acarbose (specific α-amylase inhibitor). EMMP treatment, on the other hand, resulted in remarkable regeneration and restoration of normal islet cell size. Thus, the hypoglycemic effect of EMMP could also be attributed to islets of Langerhans regeneration and resumption of insulin secretion, confirming the efficacy of phenolic compounds in the management of diabetes complications.

In contrast, hyperlipidemia presents a metabolic disorder frequently associated with type 2 diabetes.[8,18]

In this context, inhibiting dietary fat absorption is a logical target for hyperlipidemia management. Pancreatic lipase is an enzyme that plays an important role in the digestion and absorption of triglycerides in the small intestine. Triglycerides are hydrolyzed by this enzyme into glycerol and free fatty acids. As a result, natural lipase inhibitors are proposed to act as lipid-lowering agents.[27] When diabetic rats were given EMMP, their serum lipase activity was significantly lower than in untreated diabetic rats. This enzyme inhibition is supported by a significant decrease in serum and hepatic levels of Ch-T, TG, and LDL-c, accompanied by an increase in HDL-c. This backs up previous findings.[18,22,27]

Conclusions

In conclusion, EMMP administration exhibited strong hypoglycemic activity in addition to the lipid-lowering activity induced by alloxan in diabetic animals. This has clinical implications because, when used as a hypoglycemic agent, the relatively non-toxic extract of *Matricaria pubescens* can also reverse diabetes-associated dyslipidemia, which is common in diabetic patients. The restoration of serum parameters associated with carbohydrate homeostasis, such as lipase, glycemia, α-amylase, and lipid markers, demonstrates this beneficial effect. The current study has also opened up new avenues for future research, particularly in the development of a potent herbal medicine for diabetes mellitus derived from *Matricaria pubescens*.

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