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# Hypoglycaemic effect of *Calamintha officinalis* Moench. in normal and streptozotocin-induced diabetic rats

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## Abstract

The purpose of this study was to investigate the effects of a water extract from the aerial parts of *Calamintha officinalis* Moench., after either a single dose or daily oral administration for 15 days, on plasma blood glucose concentrations and basal insulin levels in normal and streptozotocin-induced diabetic rats (STZ diabetic rats). The results clearly demonstrated the hypoglycaemic effect of this plant extract in both normal and STZ diabetic rats. In addition, no changes were observed in basal plasma insulin concentrations after treatment with this plant in normal or STZ diabetic rats, indicating that the underlying mechanism of the plant's pharmacological action seems to be independent of insulin secretion. We conclude that the aqueous *C. officinalis* extract exhibits a significant hypoglycaemic effect in normal and STZ diabetic rats without affecting basal plasma insulin concentrations, and supports, therefore, its traditional use by the Moroccan population.

## Introduction

Herbal medicine has been long used for the treatment of diabetic patients and continues to be currently accepted as an alternative therapy. More than 1200 plants have been described in the scientific and popular literature as exhibiting antidiabetic properties (Marles & Farnsworth 1995; Eddouks et al 2002). The streptozotocin-induced diabetic rat (STZ diabetic rat) is considered to be a precious tool for both pathophysiological and pharmacological studies of type 1 diabetes mellitus (Burcelin et al 1995).

*Calamintha officinalis* Moench. (Lamiaceae), commonly known as calamint and locally as Mentha, is a native shrub widely distributed throughout the south-eastern region of Morocco (Tafilalet), an area in which phytotherapeutic knowledge has been and remains very developed (Eddouks et al 2002). *C. officinalis* has been used since ancient times especially for its diaphoretic, expectorant and aromatic (Nostro et al 2002), as well as antibacterial, properties (Panizzi et al 1993). In Morocco, this plant is traditionally used in treatment of hypertension and cardiovascular diseases and according to our previous ethnobotanical survey in the Fez-Boulemane region, there were 15 plant citations among 1095 patients (Jouad et al 2001). In diabetes phytotherapy, the effects of aqueous *C. officinalis* extract have never been demonstrated experimentally in either clinical or experimental type 1 diabetes mellitus. Our purpose was to analyse this ethnobotanical information to determine whether this medicinal plant contained a potential antidiabetic natural drug. We considered whether the known antihypertensive activity of *C. officinalis* could be related to the underlying mechanism of the hypoglycaemic activity. Vanadate, a potent inhibitor of tyrosine phosphatases (Tsiani et al 1998), was used as a drug reference. It is known that vanadate mimics several actions of insulin in-vivo: stimulation of hexose uptake, stimulation of lipogenesis and inhibition of lipolysis (Marti et al 2001). Administration of this compound to STZ diabetic rats normalizes plasma levels of glucose, lipids, creatinine and thyroid hormones (Heyliger et al 1985; Gupta et al 1999). Cam et al (1997) have suggested

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that vanadium-induced amelioration of the diabetic state appears to be secondary to the preservation of a functional portion of the pancreatic $\beta$-cells that initially survived after toxicity induced by streptozotocin. This modest pancreatic preservation could exert a beneficial effect on glucose homoeostasis and explains the underlying insulin-mimetic effect of vanadate in-vivo.

The objective of this study was to investigate the effect of *C. officinalis* on blood glucose levels in both normal and STZ diabetic rats. The effects of *C. officinalis* were compared with sodium vanadate as a reference hypoglycaemic drug. Finally, the effect of aqueous *C. officinalis* extract on basal plasma insulin concentrations was also analysed to determine the probable underlying mechanism of this pharmacological effect.

## Material and Methods

### Plant material

Specimens of *C. officinalis* (Lamiaceae) were collected from the Tafilalet region (semi-arid area) of Morocco in May and June 2001, and air-dried at 40°C. The plant was taxonomically identified and authenticated by Prof. M. Rejdali (Agronomy and Veterinary Institute, Rabat) and a voucher specimen (HJ 42) was deposited at the herbarium of the Faculty of Sciences and Techniques Errachidia (Eddouks et al 2002).

### Preparation of the aqueous extract

Plant material was prepared according to the traditional method used in Morocco (Eddouks et al 2002) (decoction): 1 g of powdered aerial parts, mixed with 100 mL distilled water, were boiled for 10 min and then cooled for 15 min. Thereafter, the aqueous extract was filtered using a Millipore filter (0.2 mm; Millipore, St Quentin en Yvelines, France) to remove particulate matter. The filtrates were then freeze-dried and the desired dose (mg of lyophilized aqueous *C. officinalis* extract aerial parts per kg body weight) was then prepared and reconstituted in 1.5 mL of distilled water. The yield of the freeze-dried preparation was 11%. The aqueous extract was prepared daily, just before administration. The extract obtained was then given orally to different groups of rats at a dose of 20 mg kg$^{-1}$. The dose of 20 mg kg$^{-1}$ was equivalent to that used in Moroccan traditional phytotherapy.

### Experimental design

Experiments were performed in healthy, adult male Wistar rats, 200–250 g. Rats were housed under standard environmental conditions ($23 \pm 1$°C, $55 \pm 5$% humidity, 12-h light–dark cycle) and allowed free access to water and a standard laboratory diet (carbohydrates 30%, proteins 22%, lipids 12%, vitamins 3%).

Diabetes was induced by intravenous injection of streptozotocin (Sigma, St Louis, MO) into the tail vein at a dose of 65 mg kg$^{-1}$ (Burcelin et al 1995). Streptozotocin was extemporaneously dissolved in 0.1 M cold sodium citrate buffer, pH 4.5. Eighteen hours after the streptozotocin injection, rats with fasting blood glucose levels greater than 16.5 mmol L$^{-1}$ were considered diabetic and then included in this study. The experiments were performed in diabetic rats 20 h after streptozotocin injection.

Normal and diabetic rats were randomly assigned to three different groups ($n = 6$ in each group). The control group received distilled water; treated groups received aqueous *C. officinalis* extract at a dose of 20 mg kg$^{-1}$ or the reference drug sodium vanadate (Fluka, Chemica, Switzerland) at a dose of 0.8 mg kg$^{-1}$. All experiments were performed in rats that had been fasted overnight (deprived of food for at least 12 h but allowed free access to water).

The drug solutions or vehicle were administered orally by gastric intubation using a syringe once daily at 0800 h. The effect of the vehicle, aqueous *C. officinalis* extract or vanadate on blood glucose was determined in fasted rats, 1, 2, 4 and 6 h after a single oral administration and after 2 days, 4 days, 1 week and 2 weeks of once daily repeated oral administration (20 mg kg$^{-1}$).

### Determination of parameters

Blood samples were collected from the tail vein in anaesthetized rats using ether solution inhalation. Blood glucose levels were determined by the glucose oxidase method using a reflective glucometer (Model GX; Ames Miles, Bayer Diagnostics, Genome Biotechnologies, Casablanca, Morocco). Basal plasma insulin concentrations were determined by radioimmunoassay kit (Pharmacia, Uppsala, Sweden) with a Beta matic counter (Cronex, Dupont, France). The kit included human insulin as standard and $^{125}$I-labelled human insulin antibody, which cross reacts with rat insulin.

### Statistical analysis

Data were expressed as mean $\pm$ s.e.m. Two-way analysis of variance followed by Bonferroni post tests were used to determine the significant difference within groups when compared with the start of treatment for each group. One-way analysis of variance followed by Tukey's multiple comparison test was used to determine the significant difference between groups. Differences were considered to be significant when $P < 0.05$.

## Results and Discussion

The aim of this study was to investigate the hypoglycaemic effect of aqueous extract of aerial parts of *Calamintha officinalis* in normal and STZ diabetic rats. According to our previous ethnopharmacological survey carried out in the north centre region of Morocco, *C. officinalis* was largely used for the treatment of hypertension and cardiac diseases (Jouad et al 2001), but no previous pharmacological or clinical study was carried out to test the antidiabetic property of this plant. However, it was reported that *C. machrostema*, a taxonomically related spice, possessed hypoglycaemic activity in alloxan-diabetic rats (Perez et al 1984).

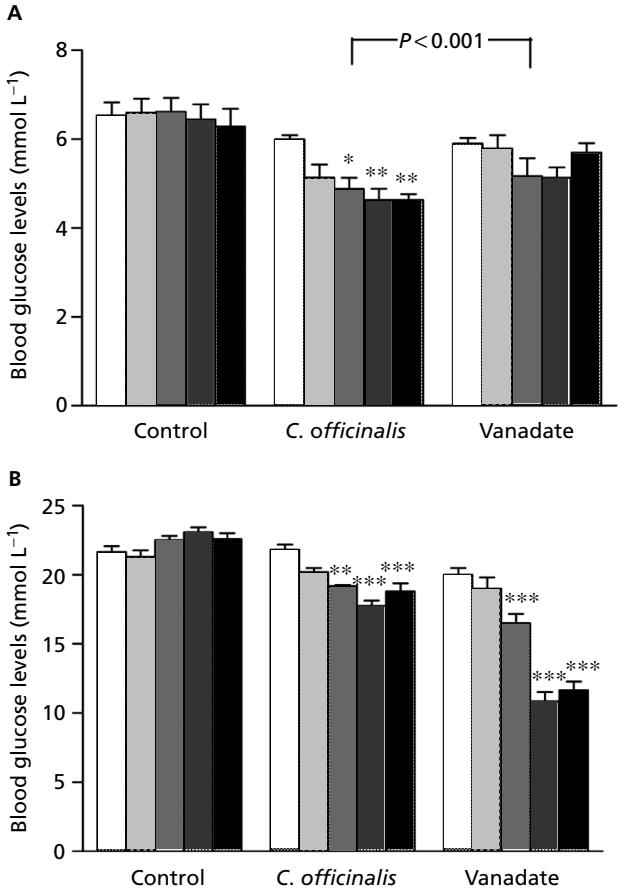


**Figure 1** Plasma glucose levels after single oral administration of aqueous *C. officinalis* extract (20 mg kg$^{-1}$) to normal (A) and streptozotocin-induced diabetic rats (B). Data are expressed as means ± s.e.m., n = 6. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, compared with baseline values. □, 0 h; ▒, 1 h; ▓, 2 h; ▓, 4 h; ■, 6 h.

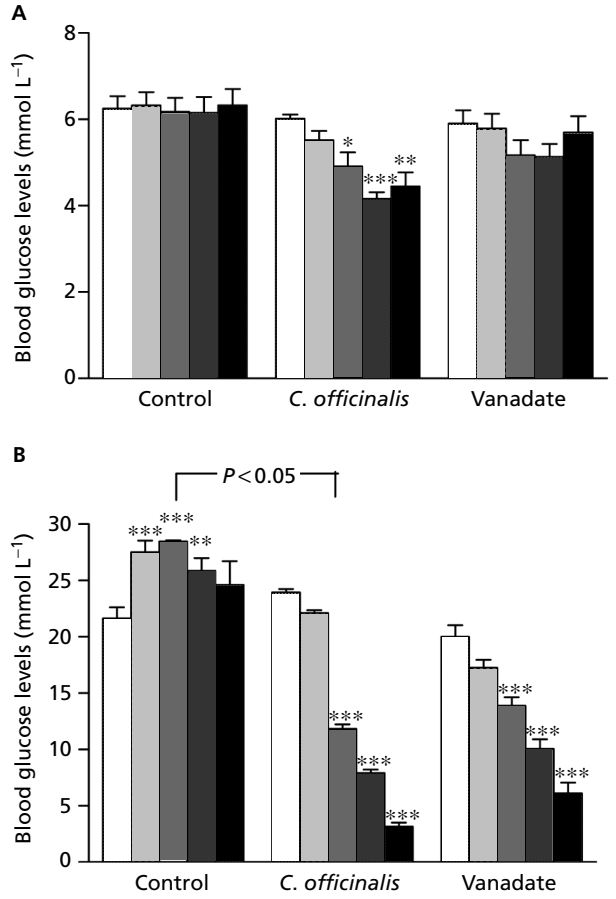


**Figure 2** Plasma glucose levels after once daily repeated oral administration of aqueous *C. officinalis* extract (20 mg kg$^{-1}$) for 15 days to normal (A) and streptozotocin-induced diabetic rats (B). Data are expressed as means ± s.e.m., n = 6. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, compared with baseline values. □, day 0; ▒, day 2; ▓, day 4; ▓, day 7; ■, day 15.

After a single oral administration, the aqueous *C. officinalis* extract (20 mg kg$^{-1}$) produced a significant decrease in blood glucose levels after 2 h when compared with the starting value ($P < 0.05$), while maximal reduction was reached 4 h after oral administration ($P < 0.01$) (Figure 1A). Vanadate (0.8 mg kg$^{-1}$) did not affect the blood glucose levels significantly. Once daily repeated oral administration of *C. officinalis* for two weeks produced, in normal rats, a potent decrease in blood glucose levels after 4 ($P < 0.05$), 7 ($P < 0.001$) and 15 days of treatment ($P < 0.01$) (Figure 2A). Vanadate treatment did not affect blood glucose levels in normal rats (Figure 1A, Figure 2A). The blood-glucose-lowering activity of *C. officinalis* in normal rats was very potent because, despite counter-regulatory factors, such as glucagon, cortisol and catecholamines, hypoglycaemia was maintained (Gelfand & DeFronzo 1984; Cryer & Gerich 1985). Our study indicates that the aqueous extract of the aerial parts of *C. officinalis* exhibits a potent and cumulative hypoglycaemic effect in normal rats after both single and repeated oral administration for 15 days.

In control diabetic rats, blood glucose levels were significantly increased from the second day ($P < 0.001$). This increment could be due to the early appearance of insulin resistance in adult STZ diabetic rats (Blondel & Portha 1989) or to glucotoxicity associated with chronic hyperglycaemia (Yki-Järvinen 1990). In STZ diabetic rats (Figure 1B), 2 h after single oral administration of *C. officinalis*, blood glucose levels dropped significantly ($P < 0.01$). Maximal reduction was observed 4 h after treatment ($P < 0.001$). However, *C. officinalis* treatment for 15 days produced a strong reduction in blood glucose levels from the 4th day ($P < 0.001$) (Figure 2B). In the second week of treatment, the blood glucose levels were very low, indicating a severe hypoglycaemic state. This finding indicates that the blood-glucose-lowering activity of extract of aerial parts of *C. officinalis* was cumulative. Vanadate treatment (0.8 mg kg$^{-1}$) caused a significant decrease in blood glucose levels from the 4th day ($P < 0.001$) to the 2nd week ($P < 0.001$) (Figure 2B).

**Table 1** Basal plasma insulin concentrations ($\mu U\,mL^{-1}$) after repeated oral administration of aqueous *C. officinalis* extract at a dose of $20\,mg\,kg^{-1}$ in normal and diabetic rats.

| Experimental group | Plasma insulin concentrations ($\mu U\,mL^{-1}$) | |
|---|---|---|
| | Day 0 | Day 15 |
| Normal rats | | |
| Control | $35.4 \pm 2.17$ | $36.45 \pm 4.12$ |
| *C. officinalis* | $32.78 \pm 0.33$ | $33.86 \pm 1.24$ |
| Vanadate | $31.81 \pm 3.15$ | $32.71 \pm 3.00$ |
| Diabetic rats | | |
| Control | $7.08 \pm 0.39$ | $6.75 \pm 0.15$ |
| *C. officinalis* | $6.22 \pm 0.32$ | $6.40 \pm 0.23$ |
| Vanadate | $6.05 \pm 0.52$ | $5.72 \pm 0.82$ |

Data are expressed as means ± s.e.m., n = 6. None of the data were significant compared with baseline values (start of treatment).

The basal plasma insulin concentrations did not differ significantly in *C. officinalis*- and vanadate-treated groups when compared with the respective baseline values in both normal and diabetic rats when treated once daily at a dose of $20\,mg\,kg^{-1}$ (Table 1).

The extract of *C. officinalis* aerial parts had no effect on basal plasma insulin concentrations in either normal or diabetic rats, indicating that this plant extract exerts a hypoglycaemic effect independently of insulin secretion. The underlying mechanism(s) of this blood-glucose-lowering activity may be due to stimulation of peripheral glucose consumption, inhibition of intestinal or renal glucose absorption (Shim et al 2003) or inhibition of endogenous glucose production (Eddouks et al 2003). *C. officinalis* treatment had a more pronounced hypoglycaemic effect in diabetic rats than in normal rats, suggesting that stimulation of peripheral glucose consumption appears to be the most probable mechanism involved in this pharmacological effect of *C. officinalis* (Silva et al 2002).

## Conclusion

We conclude that an aqueous extract of the aerial parts of *C. officinalis* was effective in decreasing blood glucose levels in normal and streptozotocin-induced diabetic rats via an extra-pancreatic action, since the basal insulin release from the $\beta$-cells of Langerhans islets did not show any significant change after *C. officinalis* treatment.

This finding supports the use of *C. officinalis* decoction by the Moroccan population for the management of diabetes mellitus. Consequently, consumption of *C. officinalis* aerial parts could prevent the complications of hyperglycaemia associated with diabetes.

Finally, the precise mechanism(s) and site(s) of this activity and the active constituent(s) of *C. officinalis* involved are still to be determined and further toxicological studies are needed.

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