**Chamaemelum nobile L. Aqueous Extract Represses Endogenous Glucose Production and Improves Insulin Sensitivity in Streptozotocin-induced Diabetic Mice**

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**Abstract:** The present study was undertaken in order to evaluate the possible mechanisms of action involved in the hypoglycaemic activity of the aqueous extract of *Chamaemelum nobile* (CN) (20 mg kg$^{-1}$). This study was carried out in Multi Low-Dose Streptozotocin-induced (MLDS) diabetic mice. Hypoglycaemic effect of CN was studied after both single and repeated oral administration of CN aqueous extract (20 mg kg$^{-1}$). Endogenous glucose production was estimated using primed-continuous 3-3H glucose infusion technique. While, euglycemic hyperinsulinemic glucose clamp technique was used to assess peripheral insulin sensitivity. Both single and repeated oral administration of aqueous CN extract evoked a prominent hypoglycaemic activity in MLDS diabetic mice. In other hand, 3-3H glucose infusion demonstrated that this hypoglycaemic activity was accompanied by a decrease in basal endogenous glucose production (EGP). EGP was lower in CS-treated group when compared to the control group, 15.5±0.5 vs 27.2±7.1 mg kg$^{-1}$ min$^{-1}$ (p<0.001) respectively. While, the metabolic clearance rate of glucose remains unchanged. In addition, we have demonstrated that CN treatment also improves insulin sensitivity in peripheral tissues, suggested by the observed higher levels of the glucose infusion rate. We can conclude that inhibition of basal endogenous glucose production and amelioration of insulin sensitivity in peripheral tissues account for the hypoglycaemic activity of aqueous CN extract in MLDS diabetic mice.

**Key words:** Endogenous glucose production, insulin sensitivity, streptozotocin, euglycemic hyperinsulinemic clamp, mice

**INTRODUCTION**

Diabetes mellitus is a complex metabolic disorder that is increasing tremendously all over the world. By 2030, the total number of people worldwide suffering from diabetes mellitus is projected to reach 366 millions[1]. Insulin resistance and excessive hepatic glucose production are two major pathophysiological abnormalities tightly associated with a cluster of diseases including diabetes mellitus, hypertension, dyslipidemia and central obesity[2-4]. Insulin resistance is defined as the inability of a known quantity of insulin to suppress hepatic glucose production and to promote glucose utilization in peripheral tissues, especially skeletal muscle and adipose tissue[5]. This insulin-resistant state seems to be due to defaults at insulin receptor and post-receptor levels[6-7]. Currently available therapeutic options for the treatment of diabetes mellitus include: dietary modification, the use of insulin or oral hypoglycaemic drugs (insulin secretagogues, insulin sensitizers and α-glucosidase inhibitors). Thiazolidinediones, are the only available drugs that reduce insulin resistance in peripheral tissues by either mimicking or enhancing insulin action without affecting β-cells insulin secreting capacity[8]. Although these therapies can control many aspects of diabetes, numerous complications are common incidents of the disease and the mortality index due to this illness continue to increase.

Plants have been used to treat diabetes mellitus since the ancient times. *Chamaemelum nobile* (CN) (Asteraceae) locally known as “Babounge”, a native shrub widely distributed throughout Morocco, is among the medicinal plants used in Moroccan folk medicine to treat a large variety of diseases including diabetes and hypertension[9]. Previously, we have reported that both single and oral repeated administration, for two weeks, of CN aerial parts aqueous extract exhibit a prominent hypoglycaemic activity in both normal and streptozotocin-induced diabetic rats[10]. The hypoglycaemic activity of CN has been attributed to the presence of Chamaemeloside, a HMG-containing...
flavonoid glucoside\textsuperscript{[11]}. However, the precise cellular and biochemical mechanisms underlying this pharmacological activity remain to be elucidated.

Studies dealing with the mode of action of potential hypoglycaemic plants will confer scientific and systematic approach for the use of these plants as hypoglycaemic agents. The present study was undertaken in order to investigate the hypoglycaemic activity of the oral administration of the aqueous extract of \textit{CN} aerial part and to investigate the possible beneficial effect the intravenous infusion of \textit{CN} extract on some pathophysiological abnormalities associated with diabetes mellitus in multi-low streptozotocin-induced (MLDS) diabetic mice, an animal model of human type 1 diabetes\textsuperscript{[12]}. In order to accomplish this goal, the effect of \textit{CN} intravenous administration on basal glucose production was studied using primed-continuous 3-\textsuperscript{H} glucose infusion\textsuperscript{[13]} while insulin sensitivity was evaluated using euglycaemic hyperglycaemic glucose clamp technique, the gold standard for assessing insulin resistance and insulin sensitivity\textsuperscript{[14]}.

\section*{MATERIALS AND METHODS}

\textbf{Plant:} Plants material of \textit{chamaemelum nobile} (Asteraceae) were collected from the Tafilalet region (semi-arid area) of Morocco in May-June 2004 and air-dried at \textit{40}\degree C. The plant was previously identified and authenticated by Pr. M. Rejdali (Agronomy and Veterinary Institute, Rabat) and a voucher specimen (ME 35)\textsuperscript{[9]} was deposited at the herbarium of the Faculty of Sciences and Techniques Errachidia.

\textbf{Preparation of the aqueous extract:} Plant material was prepared according to the traditional method used in Morocco (decoction): 1 g of powdered fruits 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 (Millipore 0.2 mm, St Quentin en Yvelines, France) to remove particulate matter. The filtrate was then freeze-dried and the desired dose (mg of lyophilized aqueous extract of \textit{CN} aerial parts per kg body weight) was then prepared and reconstituted in 1.5 mL of distilled water. The extracts obtained were then given orally to different groups of mice at a dose of 20 mg kg\textsuperscript{-1} body weight. The dose of 20 mg kg\textsuperscript{-1} was used according to the Moroccan traditional phytotherapy.

\textbf{Experimental animals:} All experiments were performed in eleven-week-old male C57BL/6J mice (Janvier, Le Genest Saint Isle, France) weighing 30±5 g. Mice were housed in a controlled environment (inverted 12-h daylight cycle, lights off at 10:00 A.M.) with free access to food and water in groups of five mice per cage at 22\degree C. All animal experimental procedures have been approved by the local ethical committee of the Rangueil hospital (Toulouse, France).

\textbf{Induction of diabetes:} Diabetes was induced in adult fasted male C57BL/6J mice by repeated intraperitoneal injection of streptozotocin (80 mg kg\textsuperscript{-1}) for four consecutive days. Streptozotocin (Sigma, St Louis, Mo, USA) was dissolved in 0.1 M fresh cold citrate buffer at pH 4.5 before use. One week after the start of injections, only mice with blood glucose levels higher than 16 mM were considered as diabetic and then included in this study.

\textbf{Acute and chronic oral treatment:} Normal and diabetic mice was randomly assigned to two groups (n = 6 in each group). The control group received distilled water and the treated group received aqueous extract of \textit{CS} at a dose of 20 mg kg\textsuperscript{-1} B.W. The \textit{CN} aerial parts aqueous extract or vehicle were administered orally by gastric intubation using a syringe once daily at 10 h a.m. The hypoglycaemic effect was evaluated in fasted mice 1, 2, 4 and 6 hours after a single oral administration and after 4, 7 and 15 days of once daily repeated oral administration.

\textbf{Surgery:} In another set of experiment and in order to perform intravenous perfusions, a catheter was implanted under anaesthesia into the femoral vein of male C57BL/6J mice. The other extremity of the catheter was tunnelled under the skin of the back, exteriorized and secured in place at the back of the neck. The mice were then allowed to recover from the surgery in individual cages.

\textbf{Endogenous glucose production:} Diabetic mice were divided randomly to two groups, control group received continuous perfusion of physiological saline solution (NaCl 0.9\%) and experimental group received continuous perfusion of \textit{CN} aqueous extract (20 mg kg\textsuperscript{-1}) at a rate of 2 \(\mu\text{L min}^{-1}\) kg\textsuperscript{-1} body weight. The mice were fasted for six hours prior to the infusions. They were connected to the infusion apparatus two hours prior to the start of the infusions with free access to water. In order to determine the rate of endogenous glucose production in mice at post-absorptive state, 3-\textsuperscript{3H}-glucose (Perkin Elmer, Boston, MA) was infused continuously at a dose of 30 \(\mu\text{Ci kg}^{-1}\text{min}^{-1}\) to ensure a detectable plasma D-(3H)\textsuperscript{3}-glucose enrichment. Plasma glucose and 3-\textsuperscript{3H}-
Blood glucose levels, single oral administration: Figure 1 depicts the blood lowering effect of a single oral administration of the aqueous extract of CS fruits (20 mg kg\(^{-1}\)) in MLDS diabetic mice. CN treatment was accompanied with an important decrease of blood glucose levels which dropped from 19.41±2.02 to 13.57±1.54 and 9.10±1.41 mM two (p<0.01) and six (p<0.01) hours after treatment respectively (Fig. 1).

Blood glucose levels; repeated oral administration: The effect of once daily repeated administration of aqueous CN extract (20 mg kg\(^{-1}\)) in MLDS diabetic mice was shown in Fig. 2. In diabetic mice treated with CN extract, blood glucose levels were decreased significantly by 39%. It dropped from 19.41±1.61-11.42±0.29 mM at the end of treatment (p<0.01) (Fig. 2).

![Fig. 1: Plasma glucose levels (mM) over 6 h after single oral administration of CN aqueous extract (20 mg kg\(^{-1}\)) in diabetic mice. Data are expressed as means ± SEM, n = 6 mice per group. *: p<0.05; **: p<0.01; ***: p<0.001 when compared to baseline values (0 h)](image-url)
Endogenous glucose production: In MLDS mice, aqueous CN extract infusion at a dose of 20 mg kg\(^{-1}\) produced a strong hypoglycaemic effect. Blood glucose levels decrease from 18.83±0.44 to 5.44±0.37 mM three hours after CN infusion (p<0.001) (Table 1). Parallel to the potent decrease in blood glucose levels, CN infusion (20 mg kg\(^{-1}\)) produced a strong decrease in endogenous glucose production (EGP). At the end of the infusion period, EGP values were significantly lower in CS treated group when compared to the control one, 15.4±0.5 vs 27.2±7.1 mg kg\(^{-1}\) min\(^{-1}\) respectively (p<0.001) (Fig. 3).

Metabolic clearance rate of glucose: There are no significant differences in basal Metabolic Clearance Rate (MCR) of glucose between CN treated diabetic group and control group (Table 1).

Euglycemic hyperinsulinemic clamp: Figure 4 shows the time-course of Glucose Infusion Rate (GIR) in CS-treated and control diabetic groups. The total amount of the infused glucose was considered to be taken up by the body tissues and, under these steady-state conditions of euglycemia and hyperinsulinemia, glucose input was equal to glucose utilization. Because a plateau GIR was achieved during the last 30 min of the clamp procedure, GIR was used as an indicator of whole body glucose utilization. Because a plateau GIR was achieved during the last 30 min of the clamp procedure, GIR was used as an indicator of whole body glucose utilization and reflects the whole body insulin sensitivity. It was significantly higher in CN treated group then in control groups, 80.72±3.47 vs 67.68±5.55±10.06 mg kg\(^{-1}\) min\(^{-1}\) respectively (p<0.05) (Fig. 4).

**DISCUSSION**

The present study was designed to investigate the hypoglycaemic activity of the aqueous extract of
Chamaemelum nobile (CN) and to evaluate the possible beneficial effect of CN infusion on hepatic glucose production and whole body insulin sensitivity in multi-
low dose streptozotocin-induced diabetic mice. Multiple injections of low doses of streptozotocin are known to affect β-cells, eliciting a subsequent immune
destruction with macrophage and lymphocyte islet infiltration, leading to β-cell lysis.[16] This animal model mimics some basic aspects of recent-onset type 1 diabetes in human patients.[12] Our results clearly
demonstrate that both single and repeated oral administration of aqueous CN extract were accompanied by an important decrease in blood glucose
levels in MLDS diabetic mice. This finding is with concordance with our previous work which has demonstrated the same prominent hypoglycaemic
activity in both streptozotocin-induced diabetic rats and normal rats.[10] Although, the cellular and biochemical mechanisms underlying this pharmacological activity
remain unknown. In order to elucidate the possible mechanism of CN hypoglycaemic effect, we have analysed the effect of aqueous CN extract perfusion in
MLDS diabetic mice. The effects of this perfusion on blood glucose levels, basal endogenous glucose production and insulin sensitivity were studied in
conscious and unrestrained diabetic mice.

Our results clearly demonstrated that continuous infusion of CN extract for three hours lowered both blood glucose levels and endogenous glucose production,
whereas the metabolic clearance rate of glucose remains unchanged. Previously, we have reported that inhibition of endogenous glucose production accounts for the hypoglycaemic activity of Spergularia purpurea aqueous extract in
streptozotocin-induced diabetic mice.[19] The liver plays a strategic role in the control of glucose homeostasis. Hepatic glucose production is determined by the rate of
hepatic glycogen breakdown, which is regulated by glucose-6 phosphatase and by the rate of hepatic gluconeogenesis, which is regulated by
phosphoenolpyruvate carboxykinase. Excessive hepatic glucose production is characteristic of untreated or poorly controlled diabetes mellitus.[18] It has been
suggested that insulinopenic state is associated with basal glucose overproduction and that increased gluconeogenesis is the main source of hepatic glucose
overproduction.[19-20] It seems to be a result of the lack of insulin repressing activity on the two key gluconeogenic enzymes, phosphoenolpyruvate
carboxykinase and glucose-6-phosphatase.[21] CN may exert its hypoglycaemic effect by repressing the activities of these key enzymes. Such metformin-like
effects have been previously reported to be related to the hypoglycaemic activity of several medicinal plants and herbal preparations.[22-26]. However, it is well known
that depletion in hepatic and muscular glycogen content is one of main metabolic features of diabetes mellitus.[27] So, it is not excluded that CN may also
prevent decrease in glycogen content especially via the activation of rate-limiting glycogenic enzyme, glycogen synthase leading to diminution in the total hepatic
glucose output. A similar effect was reported by[25] to explain the hypoglycaemic of aqueous extract of Pterocarpus marsupium in diabetic rats.

Using the euglycemic hyperinsulinemic glucose clamp technique we have confirmed that aqueous CN extract infusion improved the whole body insulin
sensitivity in MLDS diabetic mice. Streptozotocin-induced diabetes leads to the early apparition of insulin resistance in hepatic and peripheral tissues.[28,29] During
the insulin infusion rate (4 mU kg⁻¹ min⁻¹) used in this study, insulin levels of about 100 µU mL⁻¹ were achieved. At high physiological insulin levels, changes
in GIR are thought to be caused mainly by changes in the insulin receptor binding. Although we did not measure the effect of insulin on hepatic glucose
production (HGP), according to Tominaga et al., HGP can be completely suppressed under an insulin infusion rate of 3 mU kg⁻¹ min⁻¹.[30] Therefore, the GIR is
essentially synonymous with the rate of the total body glucose utilization.[31] Consequently, the results of the present study suggest that CN administration could
improve insulin sensitivity in peripheral tissues. Many herbal preparations and medicinal plants have been reported to possess similar insulin sensitizing activities
both in experimental study and clinical investigations, Indigofera myoresiensis,[32] Gymnema sylvestre,[30] Panax ginseng[33,34], Trigonella foenum-graecum[35],
Sanguis draxonis[36] and Acanthopanax senticosus[37], Gasha-jinki-gan[39,40] and dietary Guar gum.[41]. Improvement of peripheral insulin sensitivity may be a
consequence of the stimulation of insulin signalling pathway.[39,42] A similar mechanism has been reported to explain the insulin sensitizing activity of cinnamon and
fenugreek extracts.[35,39,43]. After three weeks of cinnamon treatment (300 mg kg⁻¹), the skeletal muscle insulin-stimulated IR-β and the IRS-1 tyrosine
phosphorylation levels were 18 and 33% higher in treated rats.[42]. However, the hypoglycaemic activity of fenugreek seed extract wasmediated through the stimulation of an insulin signalling pathway especially in adipocytes and liver cells.[35]. Stimulation of insulin signalling pathway in insulin sensitive tissues (liver,
muscle and adipose tissues) promotes glucose
utilization and leads to decrease blood glucose levels. It is well known that the antidiabetic thiazolidinediones reduce the insulin resistance of peripheral tissues and that this insulin sensitizing property is mediated through a subfamily of nuclear receptors, peroxisome proliferator activated receptor gamma (PPAR-γ)\(^{[43]}\). PPAR-γ receptors are found in key target tissues for insulin action, such as adipose tissue, skeletal muscle and liver and evidence indicates that these receptors are important regulators of adipocyte differentiation, lipid homeostasis and insulin action\(^{[44]}\). The reported hypoglycaemic activity of CN extract could be mediated by a beneficial effect on PPAR-γ signalling network. A similar mechanism has been demonstrated in diabetic animals treated with *Punica granatum*\(^{[45]}\) and *Pterocarpus marsupium*\(^{[46]}\) extracts. However, the precise molecular mechanism underlying this insulin sensitizing property of CN aqueous extract needs further experimental investigations to be demonstrated.

In summary, this is the first report that CN treatment decreases endogenous glucose production and improves insulin sensitivity in peripheral tissues, two major pathophysiological abnormalities associated with diabetes mellitus, in multi-low dose streptozotocin-induced diabetic mice. Our results support overall in vivo anti-hyperglycaemic activity of *Chamaemelum nobile* extract.

REFERENCES


