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Increase in insulin sensitivity by the association of chicoric acid and chlorogenic acid contained in a natural chicoric acid extract (NCRAE) of chicory (*Cichorium intybus* L.) for an antidiabetic effect

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ABSTRACT

Ethnopharmacological relevance

Chicory (*Cichorium intybus* L.) is an indigenous vegetable widely cultivated in Europe, America and Asia. In ancient times, the leaves, flowers, seeds, and roots have been used as a wealth of health benefits including its tonic effects, the ability to ease digestive problems and to detoxify liver. In Indian traditional therapy, chicory was known to possess antidiabetic effect. In the traditional medicine of Bulgaria and Italy, chicory was used as hypoglycemic decoctions.

Aims of the studies

We wanted to obtain the complete chemical composition of the natural chicoric acid extract (NCRAE), a chicory root extract rich in chicoric acid, which previously showed its glucose tolerance effect in normal rats. To investigate if the whole NCRAE is required to be effective, we performed a comparative *in vivo* experiment on STZ diabetic rats treated either with NCRAE or a mixture composed of the two major compounds of NCRAE.

Materials and Methods

¹ K. Ferrare and L.P.R. Bidel contributed equally to this study.

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LC-MS method has been used to analyze the exhaustive composition of NCRAE: we have determined that chicoric acid and chlorogenic acid represented 83.8% of NCRAE. So, we have prepared a solution mixture of chicoric acid and chlorogenic acid named SCCAM, in order to compare *in vivo* the antidiabetic effects of this last and NCRAE in streptozotocin diabetic rats. *In vitro* experiments were performed on L6 cell line both for glucose uptake and for the protective effect against H₂O₂ oxidative stress. Also, we have evaluated DPPH and ORAC (Oxygen Radical Absorbance Capacity) antioxidative capacities of the two compositions.

Results

The LC-MS analysis confirmed the high abundance of chicoric acid (64.2%) in NCRAE and a second part of NCRAE is composed of caffeoylquinic acids (CQAs) at 19.6% with among them the chlorogenic acid. This result has permitted us to prepare a mixture of synthetic L-chicoric acid (70%) and synthetic chlorogenic acid (30%): the solution is designated SCCAM. Our results showed that both NCRAE and SCCAM are able to improve a glucose tolerance in STZ diabetic rats after a subchronic administration of seven days. Alone NCRAE allows to significantly decrease the basal hyperglycemia after six days of treatment. To explain these difference of effects between NCRAE and SCCAM, we have compared their *in vitro* effects on the L6 muscle cell line both for the insulin sensitizing effect and for their protective action in pretreatment against H₂O₂. We have also compared their antioxidant capacities. In conclusion, we demonstrated that NCRAE, a natural extract of chicory (*Cichorium intybus*) rich in CRA and CQAs improves glucose tolerance and reduces the basal hyperglycemia in STZ diabetic rats.

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Cichorium

Extraction / chromatography method

Root

LC-MS

Chicoric acid 72.4 %
Caffeoyl-quinic acids 19.6 %

caffeoyle derivatives
enriched extract

Chicoric acid
70 %
Chlorogenic

In vivo experiments on
streptozotocin



- NCRAE induced an antidiabetic effect,
CGAM induced an increase in glucose

NCRAE contained a mixture of CRA, CGA and others
caffeoyle derivatives that confer this antidiabetic

Keywords

Cichorium intybus L. root extract; LC-MS analysis; Chicoric acid and chlorogenic acid; Streptozotocin diabetic rats; Antidiabetic effect; L6 cells oxidative stress assay; L6 cell glucose uptake

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1. Introduction

Type 2 diabetes (T2D) is amongst the most common metabolic diseases worldwide. It affected about 350 million people in 2011 and this number will achieve 550 million in 2030 (Whiting et al., 2011). To treat this illness, oral hypoglycemic agents including insulin secretagogues such as sulfonylureas, insulin sensitizing agents such as metformin and intestinal alpha glucosidase inhibitors such as acarbose are currently used. To restore a normal glycemia, alternative therapeutics consisting to use natural compounds extracted from plants are in studies. In traditional medicine, the chicory (*Cichorium intybus* L.) was widely used to treat diabetes in India and Pakistan (Street et al., 2013). In their review of beneficial plants used in the popular traditional medicine of Italy and Bulgaria, Leporatti and Ivancheva (2003) have reported the traditional use of *Cichorium intybus* L. decoctions for their hypoglycemic property. The antidiabetic effect of an alcoholic chicory extract in streptozotocin-induced diabetic rats have been published by Pushparaj et al. (2007) and more recently by Ghamarian et al. (2012). Often, the anti-hyperglycemic properties of the polyphenolic compounds have been described (Cheng et al., 2003; Jung et al., 2006; Namura et al., 2003; Meng et al., 2013). In our previous works, we have demonstrated the anti-hyperglycemic properties of a chicory (*Cichorium intybus* L.) root extract (natural chicoric acid extract; NCRAE) and a burdock (*Arctium lappa* L.) root (dried burdock root extract; DBRE) extract (Azay-Milhau et al., 2013; Tusch et al., 2014). These two Asteraceas extracts contain a lot of caffeoyl-derivatives. The chicory extract (NCRAE), rich in chicoric acid (dicaffeoyl tartaric acid) has a *in vitro* ability to increase glucose uptake in L6 muscular cell line (Tusch et al., 2008; Azay-Milhau et al., 2013). Moreover, *in vivo* experiments by an intraperitoneal glucose tolerance test on normal rats have brought evidence that NCRAE can improve glucose tolerance (Azay-Milhau et al., 2013). Caffeoyl derivatives, especially chicoric acid (CRA) and chlorogenic acid (CGA) are

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well documented in the literature for their antioxidant capacities (Fraisse et al., 2011; Schlernitzauer et al., 2013; Niggeweg et al., 2004; Sato et al., 2011).

In the present work, we have investigated the antidiabetic capacity of NCRAE and endeavored understand the part played by the two most abundant caffeoyl compounds contained in this extract. For that, we performed initially the identification of all caffeoyl derivatives of the extract by LC-MS analysis. On the basis of the chemical characterization of NCRAE, we have prepared a composition named SCCAM (Synthetic Chicoric and Chlorogenic Acids Mixture) containing the two major compounds of NCRAE, in proportion of 70% of synthetic L-chicoric acid (CRA) and 30% of synthetic CGA. This ratio is very close of the NCRAE CRA/CQAs ratio. We performed an *in vivo* experiment on streptozotocin (STZ) diabetic rats treated by subchronic administrations of NCRAE or SCCAM. The results have shown that the two compositions are able to improve the glucose tolerance, but only NCRAE possesses the capacity to decrease the basal hyperglycemia in animals. To understand the differences of effect between NCRAE and SCCAM, we have compared their *in vitro* insulin sensitizing effects and their protective actions in pretreatment against H₂O₂ on the L6 muscle cell line and also their antioxidant capacities.

2. Materials and methods

2.1. Biological material and drugs

Chicory (*Cichorium intybus* L.) plants were collected in Montpellier (France) and identified by botanical biologists of Botanical Institute of Montpellier (*Tela Botanica-Cichorium intybus* L., identification number: 2121). Natural chicoric acid extract (NCRAE) of roots was obtained using the method previously described (Azay-Milhau et al., 2013) with modifications. Briefly, the powder of the part of plant was poured in a cellulose cartridge and placed in a EtOH-Water (70 : 30). The concentrated crude extract was passed through an LH20 (Sigma-Aldrich, Munich, Germany) column to recover the enriched extract

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polyphenols in a EtOH-Water (40 : 60). Commercial synthetic chicoric acid (CRA) and chlorogenic acid (CGA) were purchased by Sigma-Aldrich. For L6 rat muscular cell culture, Dulbecco's Modified Eagle's Medium (DMEM) and Fetal calf serum (FCS) was provided from Biochrom (Berlin, Germany). Cocktail medium, 2 mM L-glutamine, 100 U/mL penicillin and 100 µg/mL streptomycin, were purchased by Sigma-Aldrich. For diabetes induction, streptozotocin (STZ) was also provided by Sigma-Aldrich. For LC-MS analysis, acetonitrile (Carlo-Erba, France) and formic acid (Merck, Germany) were of HPLC-grade quality. Water was permuted using a reverse osmosis Milli-Q system (Millipore, France). Chlorogenic acid, L-chicoric acid, tartaric acid, quercetin-3-O-glucuronide, kaempferol-3-O-glucuronide were provided by Sigma-Aldrich.

2.2. Chemical analysis of NCRAE by LC-MS

In a first step, the extract was characterized using a Synapt G2-S high definition mass spectrometry system (Waters Corp., Milford, MA) equipped with electrospray ionization source, to characterize elemental composition of parent and fragment ions. Chromatographic separation was carried out at a flow rate of 0.4 mL.min⁻¹ on Acquity H-Class ultrahigh performance liquid chromatography (UPLC) system (Waters Corp., Milford, MA), equipped with a Kinetex C18 100A column (100 x 2.1 mm, 2.6µm beads) from Phenomenex (France). The mobile phase consisted of permuted water (solvent A) and acetonitrile (solvent B), both phases acidified by 0.1% (v/v) formic acid. Mass spectra were acquired in the positive and negative ionization mode with a capillary voltage of 3 kV. Tandem mass spectra were acquired in Fast-DDA (Data Directed Analysis) mode so that the two most abundant ions in full scan MS would trigger tandem mass spectrometry (MS²). The TOF mass analyzer was calibrated using phosphoric acid in 1:1 (v:v) acetonitrile:H₂O from 50 to 1500 m/z to obtain mass accuracy within 3 ppm. The Synapt parameters were optimized using a chlorogenic acid standard as follows: the sample cone was set at 20 V, the source and desolvation temperature were set at 120 and 600 °C, respectively. Each sample was processed with MassLynx (V4.1) software.

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In a second step, area of the absorbance peak of each hydroxycinnamic acid was integrated at 326 nm (maximal absorbance of CGA) and expressed as equivalent absorbance of the authentic *L*-chicoric acid standard (Sigma-Aldrich, Munich, Germany). Compounds were identified by their retention times, UV absorbance spectra, and MS fragmentation pattern and numbered in conformity with the IUPAC numbering system (IUPAC, 1976). For peak assignment, we took as references LC–MS/MS characterizations from Lin and Harnly (2008), Jaiswal and Kuhnert (2011), Maruta et al. (1995). Isomers were assigned by using the appropriate standards (chlorogenic acid, caffeic acid). Since other caffeoylquinic acid derivatives were not commercially available, they were identified by comparison with chromatograms of plants belonging to Asteraceae: *Arctium lapa* L. root and shoot extracts, *Arnica montana* L. flower extract, *Cynaria scolymus* L. leaves, *Lactuca sativa* L. leaves, *Taraxacum officinale* WEB. ex WIGG. Comparison to the leaf extract from *Coffea canephora* and *Ilex paraguariensis* was also necessary for dimethoxycinnamoyl-caffeoylquinic acids (Alonso-Salces et al., 2009).

2.3. *In vivo* experiments

Experiments were performed in male Wistar rats from Charles River Laboratory (Lyon, France) and maintained on a 12h/12h light dark schedule. Institutional guidelines for animal care and use were followed and the ethical committee Languedoc-Roussillon agreed our protocol (number 101). Diabetes was obtained by intraperitoneal injection of STZ at the dose of 65 mg.kg⁻¹ in rats weighing 300-320 g and thereafter maintained in individual metabolic cages. Seven days after diabetes induction, animals were divided in three groups (four animals by group). The first group received a daily intraperitoneal (IP) administration of NCRAE at the dose of 15 mg.kg⁻¹ dissolved in saline for 7 days. The second cohort of diabetic rats were treated by IP injections for 7 days with the SCCAM (15 mg.kg⁻¹). The last group of diabetic animals received only IP saline as controls. During the treatments, we studied the time course of the following parameters: body weight, food intake, glycosuria/24 h and glycemia. At the end of the treatments, all animals were submitted to an

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oral glucose tolerance test (OGTT, glucose 3 g.kg⁻¹). Blood samples were collected from the tail vein of unfasting rats for plasma glucose determinations. For OGTT, animals were fasted for 4 h. Plasma and urinary glucose determinations were performed using the Glucose GOD-PAP kit (BIOLABO SA, France). Results are expressed as mg.dL⁻¹ for glycemia. In addition, for a better analysis of the time course of glycemia, values were calculated in % from basal values. Glycosuria evaluations were expressed as the urinary volume per 24 h x urinary glucose concentrations (g/24 h).

2.4. Determination of total polyphenol content

Total polyphenol content was determined by using the Folin-Ciocalteu reagent (Singleton and Rossi, 1965). Samples (50 µg) was diluted in a final volume of 1 mL with 0.2N of Folin-Ciocalteu reagent in water and were incubated in the dark at ambient temperature for 30 minutes. Discoloration was estimated by spectrophotometry analysis at 665 nm. Gallic acid (GA) was used as the standard for curve calibration. The total phenol content of extracts was expressed as mg of GAE (equivalents GA) per g of dry extract.

2.5. 2-Glucose uptake test on L6 cells.

L6 rat myocyte cells (LGC Promochem, France) were cultured in 12-well plates as previously described by Tousch et al. (2008). Briefly, on the day of the experiment, cells were first starved during 4 h in DMEM supplemented with 0.1% BSA and then incubated during one hour in KRB supplemented with 0.1% BSA, 5 mM glucose in the absence or in the presence of 100 nM insulin. The two compounds at 50 µg.mL⁻¹ were tested in the presence of 100 nM insulin. Cells were then gently washed and then incubated in 1 ml KRB containing 0.5 µCi [³H] deoxyglucose per well. Uptake was stopped by three washings in cold PBS and cells were lysed in 0.1N NaOH. Radioactivity was measured and total protein concentration evaluated by a Bradford method (Bradford, 1976); results are expressed in cpm . mg protein⁻¹ . 1 min⁻¹.

2.6. Survival test at an H₂O₂ oxidative stress on L6 cells.

The L6 cell culture has been managed as described above. Cells were seeded in 96-well plates (10⁴ cells per well). On day 4, the culture medium was changed by an DMEM supplemented with FCS, cocktail medium and with the different extracts at the final concentration of 50 µg.mL⁻¹ during 12 h. The next day, the cells were washed twice by 200 µL per well of Krebs-Ringer bicarbonate buffer, 0.1% BSA and then placed in the same buffer (200 µL per well) with or without H₂O₂ at 40 µM. After three hours of incubation at 37°C in a 5% CO₂ chamber, cells were washed once by PBS and incubated 5 min in 100 µL of Trypan-blue (Sigma-Aldrich) solution diluted by 3 with PBS. The trypan-blue solution was eliminated and the cell layers were visualized by Stereo Zoom Microscope for Large Fields with 16 x zoom (Axio Zoom.V16, Zeiss) with a magnification of 40%. The images were checked in and the blue color densities were calculated using ImageJ 1.48v (Image Processing and Analysis in Java: <https://imagej.nih.gov/ij/>) software (Schneider et al., 2012). The data are expressed as % of mortality.

2.7. Evaluation of the antioxidant capacities

We used both the DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) and the ORAC (Oxygen Radical Absorbance Capacity) tests. For DPPH test, we have used 100 nmoles of DPPH by assay with different quantities of samples. The decrease of absorbance at 517 nm were quantified on spectrophotometer as described by Villano et al. (2007). The free radical scavenging activity of an extracts is defined by the IC₅₀, i.e., the quantity of sample needed to obtain 50% of inhibition of the DPPH absorbance. Using quercetin standard (with an IC₅₀ of 20 µg.mL⁻¹ in our conditions), we have been also expressed the results in nmole of quercetin equivalent (QE) per mg of sample (dry material).

The ORAC test was estimated with the OxiSelect™ ORAC Assay kit (Cell Biolabs, Inc.) (Huang et al., 2002). All assays have been performed using a final concentration of

10 $\mu\text{g}\cdot\text{mL}^{-1}$ of both NCRAE and other compounds. Inhibition of 2,2'-azobis-(2-methylpropionamide) dihydrochloride (AAPH) oxidation of the antioxidant compounds led to a reduction of fluorescein fluorescence quenching measured using the Infinite 200Pro spectrofluorimeter (TECAN). Using Trolox as standard, the antioxidant activities were expressed as nmole of Trolox equivalent (TE) per mg of sample (dry material).

2.8. Data analysis

Statistical analyses were performed using analysis of variance. *In vitro* data are expressed as mean \pm SD and *in vivo* data as means \pm SEM. Difference were considered significant at $p < 0.05$ (*) or $p < 0.01$ (**) or $p < 0.001$ (***) using the Statgraphics 18[®] software.

3. Results

3.1. Chemical analysis of NCRAE content (Table 1 and 2)

Using the Folin-Ciocalteu method, we have observed that NCRAE contains essentially polyphenols (1390 mg GAE/g of dry extract). LC-MS mass spectrometry analysis has allowed us to assign and characterize these compounds: the data are shown in Table 1 and Table 2. Twenty-three peaks were separated with the column and the chromatographic gradient we used. Most of the peaks showed absorbance spectra with two bands at 220-245 nm and 315-335 nm separated by a shoulder at 290-300 nm, which were characteristic of hydroxycinnamic acids (P1 to P9, P12 to P16, P19 to P22). Four peaks exhibited absorbance spectra with two maximum bands at 254-256 nm and 352-354 nm (P10, P11, P17, P18) characteristic of flavonoids. Peaks P7, P10, P11 and P13 were unequivocally identified on the basis of their concordance with retention times, UV absorbance spectra, and MS² fragment proportions to pure authentic standards. Other peaks were tentatively assigned based on bibliographic data. Sesquiterpene lactones (three classes of bitter) previously identified in *Cichorium intybus* L. (Bais and Ravishankar, 2001; Al-Snafi, 2016) were completely missing in NCRAE. The 8-deacetylmatricarin-8-O-sulfate recently found in *Cichorium spinosum* L.

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(Brieudes et al., 2016) was not found in NCRAE. Similarly, saponins such as cynaragenins also identified in *C. intybus* were not identified in NCRAE.

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3.1.1. Assignment of hydroxycinnamic acid derivatives

Tartaric acid esters:

Peaks P1, P2, P7, P8 and P9 shared the daughter ion at m/z 149.0086 corresponding to [tartaric acid-H]⁻ fragment ion. This one gave under MS² fragmentation three fragments ions at m/z 131 [tartaric acid-H₂O-H]⁻, at m/z 103 and at m/z 87. Peak P1 and P2 m/z 311.0407 [M-H]⁻ yielded MS² fragment ions at m/z 179 [caffeic acid-H]⁻ and m/z 135 [caffeic acid-CO₂-H]⁻, and were identified as caffeoyl-*O*-tartaric acid isomers, also named caftaric acid isomers. Peak 2 predominantly formed the adduct m/z 623 [2 caftaric acid -H]⁻ in MS¹ experiment, already observed by Schütz et al. (2005) for the *trans* isomer. Consequently, peak 1 was consistent with the *cis*-caftaric acid and peak 2, with the *trans*-caftaric acid. Peaks 7, 8, 9 exhibited absorbance spectra shapes similar to *trans*-chicoric acid (*L*-chicoric acid) with the pseudo-molecular ion m/z 473.0699 of dicaffeoyltartaric acid esters and exhibited daughter ions at m/z 311, 293, 179, 149. They corresponded to m/z 311 [caffeoyltartaric acid-H]⁻, 293 [caffeoyltartaric acid-H₂O-H]⁻, 179 [caffeic acid-H]⁻, 149 [tartaric acid-H]⁻. This fragmentation behavior was in accordance with previous reports (Bergeron et al., 2002; Schultz et al., 2005). Peak 7 was positively identified as *L*-chicoric acid using pure standard. P7 is the preponderant compound of the extract. Peaks 8 and 9 may correspond to its two other diastereoisomers, *D*-chicoric acid and *meso*-chicoric acid described by Buiarelli et al. (2010) and Carazzone et al. (2013). Similarly to Khoza et al. (2016), we observed that the fragmentation pattern of the three isomers is tightly similar in electrospray ionization conditions. Peak 9 was attributed to the *meso* isomer because of its lower amount of fragment m/z 293 previously described (Llorach et al., 2008). No guarantee can be given that the formation of these two later isomers was not occurring during extraction, elution or electrospray ionization processes. Peaks 8 and 9 are in much reduced amount compared to *L*-

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chicoric acid. Peak P6 exhibited a parent ion m/z 491.0794, which formed in MS^2 experiment, two fragment ions at m/z 293 and m/z 329, and a fragmentation pattern already found for a tartaric acid derivative of root and herb juice of *Taraxacum officinale* Web. Ex Wigg. (Schultz et al., 2005) and root extract of *Taraxacum formosanum* Kitam (Chen et al., 2012). Presence of fragment ion m/z 329 indicated the loss of a caffeic moiety, which was confirmed by presence of fragment ions m/z 179 [caffeic acid-H]⁻ and m/z 135 [caffeic acid-CO₂-H]⁻. MS^3 fragmentation of ion m/z 329 yielded m/z 311 [caffeoyltartaric acid-H]⁻, m/z 149 [tartaric acid-H]⁻ and m/z 135 [caffeic acid-CO₂-H]⁻. Presence of fragment m/z 293 was attributed to the loss of dihydroxyphenyllactic acid plus H₂O [M- dihydroxyphenyllactic acid - H₂O-H]⁻ by Schütz et al. (2005) and Chen et al. (2012). In addition, peak 6 had similar absorbance spectrum to peak 8 previously described (Schultz et al., 2005) for dandelion (246 nm, 300 sh, 332 nm). The minor peak P6 was consistent with caffeoyl-dihydroxyphenyllactoyltartaric acid.

Caffeoylquinic acid esters :

Peaks 12, 13, 14, 16 and 19 exhibited absorbance spectra shapes similar to chlorogenic acid with the daughter ion at m/z 191.0563 corresponding to [quinic acid-H]⁻. This one gave under MS^n fragmentation two fragments ions at m/z 127 [quinic acid-CO-2H₂O-H]⁻ and m/z 173 [quinic acid-H₂O-H]⁻. Monocaffeoylquinic acids (parent ion of m/z 353.0877) and dicaffeoylquinic acids (parent ion of m/z 515.1194) were assigned using the identification hierarchical key of quinic acid derivatives proposed by Clifford et al. (2006). Peaks 12 and 16 displayed a fragment ion at m/z 173 [quinic acid-H₂O-H]⁻ base peak, characteristic of the caffeoyl group esterified to the 4-OH position of quinic acid. When caffeoyl groups are esterified to the 3-OH position and 5-OH position of quinic acid, the m/z 191 [quinic acid-H]⁻ fragment ion remains the base peak and the signal of m/z 179 [caffeic acid-H]⁻ fragment ion is more significant for 3-OH acylylated position of the quinic acid (Peak 13 and 14). The minor peak P19 had a parent ion m/z 615.1428 corresponding to C₂₉H₂₈O₁₅ and had 3 daughter ions, m/z 515 [dicaffeoylquinic acid-H]⁻, m/z 353 [caffeoylquinic acid-H]⁻, and m/z 453 suggesting

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the fragment [caffeoyl-succinoylquinic acid-H]⁻. We observed the presence of a secondary peak at m/z 335 [caffeoylquinic acid-H₂O-H]⁻ in the MS² spectrum, which indicated that the succinoyl group is esterified at the 4-acyl chlorogenic acid (Jaiswal and Kuhnert, 2011). We found that P19 correspond to 1,5-di-O-caffeoyl-3-O-succinoylquinic acid previously described in *Arctium lapa* L. by Tusch et al. (2014). Peak 20 and 21 shared a pseudo-molecular ion m/z 543.1508 [M-H]⁻ corresponding to C₂₇H₂₈O₁₂ characteristic of dimethoxycinnamoyl-caffeoylquinic acids (Alonso-Salces et al., 2009), also referred as methylferuloyl-O-caffeoylquinic acid (Jaiswal and Kuhnert, 2011). They shared the secondary ion m/z 381.1191 [M-H]⁻, corresponding to the loss of the methylferuloyl moiety. This is confirmed in MS² experiment by the appearance of fragment ions m/z 207 [dimethoxycinnamic acid-H]⁻ and m/z 103 [dimethoxycinnamic acid-H-H₂O-2CH₃[•]-2CO]⁻. The absence of the secondary ion m/z 349 indicated that the second acyl group esterified on quinic acid is not a dihydroxy-methoxy-cinnamic acid residue. Therefore, according to the identification key based on the fragmentation pattern of dimethoxycinnamoyl-caffeoylquinic acids in Collision Induced Dissociation-MS-MS, established by Alonso-Salces et al. (2009), peaks 20 and 21 were tentatively assigned 3-O-methylferuloyl-4-O-caffeoylquinic acid and 3-O-methylferuloyl-5-O-caffeoylquinic acid, respectively.

3.1.2. Assignment of flavonoids and coumarins.

Analysis by MS² fragmentation of peaks P10 and P17 resulted in a common ion at m/z 301.0353. Its MS³ fragmentation gave 4 fragments: m/z 271 ([M-H-CH₂O]⁻), m/z 255 ([M-H-H₂O-CO]⁻), m/z 179 ([^{1,2}A-H]⁻) and ions at m/z 151 (^{1,2}A-CO), originating from a retro-Diels-Alder (RDA) reaction similarly to that from the fragmentation of a standard solution of quercetin. We observed that Peak P10 had a parent ion m/z 477.0644 [M-H]⁻ and that its fragment ion m/z 301 corresponded to the loss of the glucuronide moiety (176 amu). In MS² experiment, P10 conformed to fragmentation of quercetin-3-O-glucuronide, since quercetin-4-O-glucuronide and quercetin-7-O-glucuronide have additional characteristic fragments not found in this extract (Davis et al., 2006). Therefore, peak P10 was assigned to quercetin-3-O-

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glucuronide. Peak P17 had a parent ion m/z 505.0994 $[M-H]^-$ and yield to m/z 463 in MS^2 and the loss of 206 amu, which indicates a hexoside moiety (162 amu) linked to an acetyl group (44 amu). It could correspond to quercetin-7-*O*-(6''-acetyl-glucoside) since it has already been found in *Cichorium* (Llorach et al., 2008) and Lettuce (Ribas-Agusti et al., 2011). Analysis by MS^2 fragmentation of peaks P11 and P18 resulted in the ion at m/z 285.0435. Fragmentation of the ion at m/z 285 gave a fragment ion at m/z 255 (loss of 30 amu, $[Y_0^- -CH_2OH]^-$) which is characteristic of kaempferol. The loss of 176 amu in MS^2 experiment by Peak P11 suggests the loss of glucuronide moiety. Peak P11 was assigned to kaempferol-7-*O*-rutinoside and positively confirmed by its authentic standard. Peak P18 had a pseudo-molecular ion m/z 593.1511 $[M-H]^-$, producing secondary ions m/z 285 $[kaempferol-H]^-$ and m/z 447 $[(kaempferol+hexose)-H]^-$, It was assigned to an undetermined kaempferol-*O*-hexosyl-rhamnoside. Peak P22 had a parent ion m/z 339.0650 $[M-H]^-$ corresponding to $C_{15}H_{15}O_9$, and the daughter ions at m/z 177.0193 ($C_9H_5O_4$) corresponding to the loss of hexoside moiety. The MS^2 experiment yielded to m/z 133.0301 $[esculetin-H]^-$. Peak 22 was assigned to esculin and positively identified using authentic standard. P22 is a common coumarin in *Cichorium* species (Kisiel and Michalska, 2002) also name cichoriin.

In summary, NCRAE is essentially composed of 72.4% of caffeoyl tartaric acids with chicoric (dicaffeoyls) acids that represent 64.2% of the extract and 19.6% of caffeoyl quinic acids (mono and diCQAs) among which 2.1% of methylferuloyl-*O*-caffeoylquinic acid. The rest of the NCRAE content is flavonoïds derivatives (6.6%). These results have led to the preparation of the SCCAM solution combining 70% of CRA and 30% of CGA, close to the 0.3 CQAs/CRA ratio of NCRAE.

3.2. In vivo experiments.

3.2.1. Evidence of the obtaining of a severe diabetic state

Seven days after STZ injection in rats, glycemia raised from 108 ± 3 to 561 ± 21 mg. dL^{-1} and plasma insulin dropped from 3.78 ± 0.37 to 0.38 ± 0.04 $ng.mL^{-1}$ i.e. a loss of 90%.

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3.2.2. Time course of the physiological parameters

The first consequences of the diabetes were a stop in the gain of body weight and a clear increase in food intake ($p < 0.01$) (Fig. 1). The daily administration of NCRAE or SCCAM did not modify these evolutions (Fig. 1A, 1B). After STZ injection, glycosuria appeared and raised to high values for all animals. NCRAE or SCCAM treatments induced a significant stabilization (Fig. 1C). So at the 6th day glycosurias of treated rats were 12.9 ± 0.6 and 14.3 ± 0.5 g/24 h respectively under NCRAE or SCCAM administrations versus 18.5 ± 1.3 g/24 h for control diabetic animals ($p < 0.01$).

3.2.3. Effect on blood glucose levels

Time course of hyperglycemia.

In control diabetic rats, we observed a continuous hyperglycemia of about 600 mg.dL^{-1} (Fig. 2). NCRAE treatment induced a clear decrease in hyperglycemia from the 2th to the 6th day ($433 \pm 33 \text{ mg.dL}^{-1}$) ($p < 0.01$). In contrast SCCAM treatment did not significantly change the time course of hyperglycemia (Fig. 2A and 2B). Thus, we only observed a reduction of hyperglycemia with NCRAE administration (-18% on the 6th day, $p < 0.01$) (Fig 2B).

Oral glucose tolerance test (OGTT) (Fig. 3).

The oral charge in glucose induced a clear increase in hyperglycemia in all diabetic animals. However, this increase is slight during the first 30 minutes with SCCAM. From 90 minutes a more rapid decrease in hyperglycemia is observed in both groups of treated rats. So at 180 minutes hyperglycemia was 412 ± 17 and $415 \pm 17 \text{ mg.dL}^{-1}$ respectively, for NCRAE and SCCAM treated animals *versus* $530 \pm 40 \text{ mg.dL}^{-1}$ for control diabetic rats ($p < 0.001$).

3.3. *In vitro* experiments (Fig.4).

3.3.1. Insulin sensitizing investigations on L6 cells.

The results were reported on the Fig.4A: As expected, insulin (100 nM) increased basal glucose uptake in L6 cells. Addition of NCRAE significantly increased glucose uptake at 50

mg.mL⁻¹ (p<0.05) which is in agreement with our previous report (Tousch et al., 2008). At the same concentration of 50 µg.mL⁻¹, SCCAM solution has also led to an increase of the glucose uptake (p<0.01) with a value that is close to the NCRAE values.

3.3.2. Oxidative stress (H₂O₂) survival test on L6 cells.

L6 cells were submitted at a pretreatment with NCRAE or SCCAM during 12 hours after that the compounds are eliminated by two washes. Then, 40 µM H₂O₂ oxidative treatment are applied. The mortality of the cells is quantified using a vital trypan blue dye (Fig.4B). In these conditions, the SCCAM pretreatment did not have a significant protective effect while NCRAE pretreatment induced a low but significant protective effect with a mortality of 46.8 ± 3.6 % comparatively in the control at 55.4 ± 3.6 % (p<0.05).

3.4. Quantification of the antioxidant capacities.

NCRAE exhibited a DPPH-free radical scavenging with a IC₅₀ at 61.4±1.75 µg.mL⁻¹ and a QE.mg⁻¹ at 1077 ± 31 nmoles compared at SCCAM with a IC₅₀ at 29.6±1.2 µg.mL⁻¹ and a QE.mg⁻¹ at 2240 ± 91 nmoles. The ORAC (Oxygen Radical Absorbance Capacity) test results were similar to those of DPPH tests with a TE at 3619 ± 48 nmoles.mg⁻¹ for NCRAE and 7020 ± 69 nmoles.mg⁻¹ for SCCAM. So, the chemical antioxidant capacity of SCCAM is clearly higher than NCRAE.

4. Discussion

The present study demonstrates that NCRAE, a natural caffeoyl derivatives extract, is able to reduce basal hyperglycemia and to improve oral glucose tolerance in streptozotocin diabetic rats, pointing out that NCRAE may be considered of interest for the treatment of diabetes.

The chemical analysis by LC-MS has allowed to obtain interesting information on the NCRAE composition, essentially composed of caffeoyltartaric acid isomers with *L*-chicoric acid (64.2%) and its other isomers and derivatives esters of caffeoylquinic acids (CQAs) (19.6%). The presence of CQAs under mono and di-caffeoylquinic acids forms has been previously described by Milala et al. (2009). The high content of chicoric acid rather

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described in lower quantity in roots than in the aerial parts of chicory (Al-Snafi et al., 2016; Brieudes et al., 2016) shows that the chromatography procedure we used allows to concentrate the caffeoyl derivatives. The presence of quercetin-3-*O*-glucuronide in NCRAE was described in the roots of chicory (Schultz et al., 2005). We can also note the presence of Ferulic acid derivatives at a lower proportion (2.1%).

STZ diabetes induced rat model is known as an appropriate model to search for potential therapies since it mimics several metabolic disorders observed in human diabetes (Urgate et al., 2012). In our STZ model, the increase in food intake and the absence in body weight gain are in agreement with the previous report (Akbarzadeh et al., 2007). STZ injection induced a severe diabetes, i.e., insulinemia dramatically dropped (-90%) due to the fall in the pancreatic insulin content (Masiello et al., 1998). In these conditions, the antidiabetic effect of NCRAE cannot be the consequence of a pancreatic insulinotropic action previously reported on INS1 cells (Tousch et al., 2008). One possibility to explain the antidiabetic property of NCRAE could be an insulin sensitizing effect on peripheral tissues as muscles. Indeed, we have previously demonstrated that NCRAE has the ability *in vitro* to enhance glucose uptake in L6 muscle cell line and does not act on hepatic glucagon-induced glycogenolysis *via* hepatic glucose 6 phosphatase activity (Azay-Milhau et al., 2013). The results on L6 muscular cells are in agreement with the report of Lee et al. (2007) on the same *in vitro* model. Here, we have shown that SCCAM possess a similar capacity that NCRAE to enhance the glucose uptake on the L6 cells, indicating the value of the mixture of chicoric acid and chlorogenic acid on insulin sensitivity. The presence of Ferulic acid derivatives in NCRAE can be beneficial since *trans*-Ferulic acid has been reported to exert an antidiabetic effect by modulating insulin signaling pathway in the liver of type 2 diabetic rats (Narasimhan et al., 2015). The delayed improvement in OGTT can be due to the insulin sensitizing action of NCRAE by caffeoyltartaric acid derivatives and the stabilization of glycosuria may be related to the decrease in basal hyperglycemia.

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Regarding the SCCAM treatment, it did not change basal hyperglycemia in STZ diabetic rats.

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However an immediate and clear improvement in OGTT was observed. The capacity of CGA to enhance glucose uptake in peripheral tissues and to reduce hepatic glycogenolysis (Meng et al., 2013) by inhibition of glucose 6-phosphatase activity (Hemmerle et al., 1997) can explain the stabilization in glycosuria and OGTT improvement with SCCAM treatment. Moreover, increasing evidence showed that oxidative stress participates in pathogenesis of diabetes (Maritim et al., 2003; Msolly et al., 2013; Nikolic et al., 2014). So, it has been necessary to consider the antioxidant capacities of the two products. For their antioxidant potential, the two compositions revealed significant activities, around twice for SCCAM. Consequently, a direct antioxidant effect cannot be responsible of the basal glycemia decrease observed only with NCRAE. In regard to the capacity of cell protection against H₂O₂ oxidative stress, the treatment of 12 hours with NCRAE induced a small decrease of mortality while no effect are observed with SCCAM. Moreover in our experiments conditions, the chemical antioxidant capacity does not have any correlation with the cellular protective capacity of these compositions against H₂O₂.

Through a better control of hyperglycemia known to induce oxidative stress (Esposito et al., 2002; Ceriello and Testa, 2009), NCRAE could have a protective effect on the evolution course and complications of diabetes.

In conclusion, we demonstrated that NCRAE, a natural extract rich in CRA from chicory (*Cichorium intybus*) improves glucose tolerance and reduces the basal hyperglycemia in STZ diabetic rats. These interesting effects of NCRAE are the result of the association of CRA and CGA but also at the presence of other hydroxycinnamic acids.

Declaration of interest

The authors declare no conflict of interest and no financial interference with the IP rights of the indigenous people.

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Author Contributions

D.T., K.F. and L.P.R.B. designed the research. L.P.R.B. and G.C. performed the LC-MS analysis. K.F. and A.A. performed the *in vitro* and *in vivo* experiments. P.P. and J.A.-M. performed the *in vivo* experiment. D.T., P.P. and A.D.L. analyzed the data and wrote the manuscript. All authors reviewed the manuscript.

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REFERENCES

- Akbarzadeh, A., Narouzian, D., Mehrabi, M.R., Jamshidi, Sh., Farhangi, A., Allah Verdi, A., Mofidian, S. M. A., Lame Rad, B., 2007. Induction of diabetes by streptozotocin in rats. *Indian J. Clin. Biochem.* 22, 60-64.
- Alonso-Salces, R.M., Guillou, C., Berrueta, L.A., 2009. Liquid chromatography coupled with ultraviolet absorbance detection electrospray ionization, collision induced dissociation and tandem mass spectrometry on a triple quadrupole for the on-line characterization of polyphenols and methylxanthines in green coffee beans. *Rapid Commun. Mass Spectrom.* 23, 363-383.
- Al-Snafi, A.E., 2016. The contents and Pharmacological Importance of *Corchorus capsularis*: A Review. *Chemist. Res. J.* 1, 9-16.
- Azay-Milhau, J., Ferrare, K., Leroy, J., Aubaterre, J., Tournier, M., Lajoix, A.-D., Tousch, D., 2013. Antihyperglycemic effect of a natural chicoric acid extract of chicory (*Cichorium intybus* L.): A comparative in vitro study with the effects of caffeic and ferulic acids. *J. Ethnopharm.* 150, 755-760.

Comment citer ce document :

Ferrare, K., Bidel, L., Awwad, Poucheret, Cazals, G., Lazennec, Azay-Milhau, J., Tournier, Lajoix, Tousch, D. (Auteur de correspondance) (2018). Increase in insulin sensitivity by the association of chicoric acid and chlorogenic acid contained in a natural chicoric acid extract (NCRAE) of chicory (*Cichorium intybus* L.) for an antidiabetic effect. *Journal of Ethnopharmacology*.

Bais, H. P., Ravishankar G. A., 2001. *Cichorium intybus* L. - cultivation, processing, utility, value addition and biotechnology, with an emphasis on current status and future prospects. J. Sci. Food Agric. 81, 467-484.

Bergeron, C., Gafner, S., Batcha, L.L., Angerhofer, C. K., 2002. Stabilization of caffeic acid derivatives in *Echinacea purpurea* L. glycerin extract. J. Agric. Food Chem. 50, 3967-3970.

Bradford, M.M., 1976. A dye binding assay for protein. Anal. Biochem. 72, 248-254.

Brieudes, V., Angelis, A., Vougianniopoulou, K., Pratsinis, H., Kletsas, D., Mitakou, S., Halabalaki, M.; Skaltsounis, L.A., 2016. Phytochemical analysis and antioxidant potential of the phytonutrient-rich decoction of *Cichorium spinosum* and *C. intybus*. Planta Med. 82, 1070-1078.

Buiarelli, F., Coccioli, F., Merolle, M., Jasionowska, R., Terracciano, A., 2010. Identification of hydroxycinnamic acid-tartaric acid esters in wine by HPLC-tandem mass spectrometry. Food Chem. 123, 827-833.

Carazzone, C., Mascherpa, D., Gazzani, G., Papetti, A., 2013. Identification of phenolic constituents in red chicory salads (*Cichorium intybus*) by high-performance liquid chromatography with diode array detection and electrospray ionization tandem mass spectrometry. Food Chem. 138, 1062-1071.

Ceriello, A., Testa, R., 2009. Antioxidant anti-inflammatory treatment in type 2 diabetes. Diabetes Care. 32 Suppl 2, S232-S236.

Chen, H.J., Inbaraj, B.S., Chen, B.H., 2012. Determination of phenolic acids and flavonoids in *Taraxacum formosanum* Kitam by liquid chromatography-tandem mass spectrometry coupled with a post-column derivatization technique. Int. J. Mol. Sci. 13, 260-285.

Comment citer ce document :

Ferrare, K., Bidet, L., Awwad, Poucheret, Cazals, G., Lazennec, Azay-Milhau, J., Tournier, Lajoix, Tusch, D. (Auteur de correspondance) (2018). Increase in insulin sensitivity by the association of chicoric acid and chlorogenic acid contained in a natural chicoric acid extract (NCRAE) of chicory (*Cichorium intybus* L.) for an antidiabetic effect. Journal of Ethnopharmacology.

Release of beta-endorphin by caffeic acid to lower plasma glucose in streptozotocin induced diabetic rats. *Horm. Metab. Res.* 35, 251-258.

Clifford, M.N., Knight, S., Surucu, B., Kuhnert, N., 2006. Characterization by LC-MSⁿ of four new classes of chlorogenic acids in green coffee beans: dimethoxycinnamoylquinic acids, diferuloylquinic acids, caffeoyl-dimethoxycinnamoylquinic acids and feruloyl-dimethoxycinnamoylquinic acids. *J. Agric. Food Chem.* 54, 1957-1969.

Davis, B.D., Needs, P.W., Kroon, P.A., Brodbelt, J.S., 2006. Identification of isomeric flavonoid glucuronides in urine and plasma by metal complexation and LC-ESI-MS/MS. *J. Mass Spectrom.* 41, 911-920.

Esposito, K., Nappo, F., Marfella, R., Giugliano, G., Giugliano, F., Ciotola, M., Quagliaro, L., Ceriello, A., Giugliano, D., 2002. Inflammatory cytokine concentrations are acutely increased by hyperglycemia in humans: role of oxidative stress. *Circulation.* 106, 2067-2072.

Fraisse, D., Felgines, C., Texier, O., Lamaison, J.L., 2011. Caffeoyl derivatives major antioxidant compounds of some wild herbs of the *Asteraceae* family. *Food Nutr. Sci.* 2, 181-192.

Ghamarian A., Abdollahi M., Su X., Amiri A., Ahadi A., Nowrouzi A., 2012. Effect of chicory seed extract on glucose tolerance test (GTT) and metabolic profile in early and late stage diabetic rats. *Daru.* 20, 56-64.

Hemmerle, H., Burger, H.J., Below, P., Schubert, G., Rippel, R., Schindler, P.W., Paulus, E., Herling, A.W., 1997. Chlorogenic acid and synthetic chlorogenic acid derivatives: novel inhibitors of hepatic glucose-6-phosphate translocase. *J. Med. Chem.* 40, 137-145.

Huang, D., Ou, B., Hampsch-Woodill, M., Flanagan, J.A., Deemer, E.K., 2002. Development and validation of oxygen radical absorbance capacity assay for lipophilic antioxidants

Comment citer ce document :

IUPAC Commission on the Nomenclature of Organic Chemistry (CNOC) and IUPAC-IUB Commission on Biochemical Nomenclature (CBN), 1976. Nomenclature of cyclitols. Recommendations. *Biochem J.* 153, 23-31.

Jaiswal, R., Kuhnert, N., 2011. How to identify and discriminate between the methyl quinates of chlorogenic acids by liquid chromatography-tandem mass spectrometry. *J. Mass Spectrom.* 46, 269-281.

Jung, U.J., Lee, M.K., Park, Y.B., Jean, S.M., Choi, M.S., 2006. Antihyperglycemic and antioxidant properties of caffeic acid in db/db mice. *J. Pharma. Exp. Ther.* 318, 476-483.

Khoza, B.S., Gbashi, S., Steenkamp, P.A., Njobeh, P.B., Madala, N.E., 2016. Identification of hydroxycinnamoyl tartaric acid esters in *Bidens pilosa* by UPLC-tandem mass spectrometry. *S. Afr. J. Bot.* 103, 95-100.

Kisiel, W., Michalska, K., 2002. A new coumarin glucoside ester from *Cichorium intybus*. *Fitoterapia.* 73, 544-546.

Lee, E.S., Uhm, K.O., Lee, Y.M., Han, M., Lee, M., Park, J.M., Suh, P.G., Park, S.H., Kim, H.S., 2007. CAPE (caffeic acid phenethyl ester) stimulates glucose uptake through AMPK (AMP-activated protein kinase) activation in skeletal muscle cells. *Biochem Biophys Res Commun.* 361, 854-858.

Leporatti, M.L., Ivancheva, S., 2003. Preliminary comparative analysis of medicinal plants used in the traditional medicine of Bulgaria and Italy. *J Ethnopharmacol.* 87, 123-142.

Lin, L.-Z., Harnly, J.M., 2008. Identification of hydroxycinnamoylquinic acids of *Arnica* flowers and burdock roots using a standardized LC-DAD-ESI/MS profiling method. *J. Agric. Food Chem.* 56, 10105-10114.

Characterisation of polyphenols and antioxidant properties of five lettuce varieties and escarole. *Food Chem.* 108, 1028-1038.

Maruta, Y., Kawabata, J., Niki, R., 1995. Antioxidative caffeoyl-quinic acid derivatives in the roots of burdock (*Arctium lappa* L.). *J Agric Food Chem.* 43, 2592-2595.

Maritim, A.C., Sanders, R.A., Watkins, J. B., 2003. Diabetes, oxidative stress, and antioxidants: A review. *J. Bioch. Molecul. Toxicol.* 17, 24-38.

Masiello, P., Broca, C., Gross, R., Roye, M., Manteghetti, M., Hillaire-Buys, D., Novelli, M., Ribes G., 1998. Experimental NIDDM. Development of a new model in adult rats administered streptozotocin and nicotinamide. *Diabetes.* 47, 224-229.

Meng, S., Cao, J., Feng, Q., Peng, J., Hu, Y., 2013. Roles of Chlorogenic acid on Regulating Glucose and Lipids Metabolism: A review. *Evid Based complement Alternat Med.*, 2013, 801457. <http://dx.doi.org/10.1155/2013/801457>.

Milala, J., Grzelak, K., Król, B., Juśkiewicz, J., Zduńczyk, Z., 2009. Composition and properties of *Chicorium* extracts rich in fructans and polyphenols. *Pol. J. Food Nutr. Sci.* 59, 35-43.

Msolly, A., Miled, A., Kassab, A., 2013. Hydrogen peroxide: an oxidant stress indicator in type 2 diabetes mellitus. *JCvD.* 1, 48-52.

Namura, E., Kashiwada, A., Hasada, A., Nakamura, K., Marishita, X., Tsimo, T., Tamiguchi, H., 2003. Synthesis of amide compounds of ferulic acid and their stimulatory effects on insulin secretion *in vitro*. *Bioorg. Med. Chem.* 11, 3807-3813.

Narasimhan, A., Chinnaiyan, M., Karundevi, B., 2015. Ferulic acid exerts its antidiabetic effect by modulating insulin-signaling molecules in the liver of high-fat diet and fructose-induced type-2 diabetic adult male rat. *Appl. Physiol. Nutr. Metab.* 40, 769-781.

Nikolic, D., Stanimirovic, J., Bjelogrić, P., Isenovic, E.R., 2014. Oxidative stress and the role of antioxidative treatment in diabetes mellitus. *Oxid. Antioxid. Med. Sci.* 3, 9-14.

Pushparaj PN, Low HK, Manikandan J, Tan BK, Tan CH. 2007. Anti-diabetic effects of *Cichorium intybus* in streptozotocin-induced diabetic rats. *J Ethnopharmacol.* 111, 430-434.

Ribas-Agustí, A., Gratacós-Cubarsí, M., Sárraga, C., García-Regueiro, J.A., Castellari, M., 2011. Analysis of eleven phenolic compounds including novel p-coumaroyl derivatives in lettuce (*Lactuca sativa* L.) by ultra-high-performance liquid chromatography with photodiode array and mass spectrometry detection. *Phytochem. Anal.* 22, 555-563.

Sato, Y., Itagaki, S., Kurokawa, T., Ogura, J., Kobayashi, M., Hirano, T., Sugawara, M., Iseki, K., 2011. *In vitro* and *in vivo* antioxidant properties of chlorogenic acid and caffeic acid. *Int. J. Pharm.* 403, 136-138.

Schlernitzauer, A., Oiry, C., Hamad, R., Galas, S., Cortade, F., Chabi, B., Casas, F., Pessemesse, L., Fouret, G., Feillet-Coudray, C., Cros, G., Cabello, G., Magous, R., Wrutniak-Cabello, C., 2013. Chicoric acid is an antioxidant molecule that stimulates AMP Kinase pathway in L6 myotubes and extends lifespan in *Caenorhabditis elegans*. *PLoS ONE.* 8, e78788.

Schneider, C.A., Rasband, W.S., Eliceiri, K.W., 2012. NIH Image to ImageJ: 25 years of image analysis. *Nat. Methods.* 9, 671- 675.

Schultz, K., Kammerer, D.R., Carle, R., Schieber A., 2005. Characterization of phenolic acids and flavonoids in dandelion (*Taraxacum officinale* Web. ex Wigg.) root and herb by high-performance liquid chromatography/electrospray ionization mass spectrometry. *Rapid Commun. Mass Spectrom.* 19, 179-186.

Comment citer ce document :

Singleton, V.L. and Rossi, J.A., 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American Journal of Enology Viticulture. 16, 144-158.

Street, R.A., Sidana, J., Prinsloo, G., 2013. *Cichorium intybus*: Traditional Uses, Phytochemistry, Pharmacology, and Toxicology. Evid Based Complement Alternat Med., 2013, 579319. doi: 10.1155/2013/579319.

Tousch, D., Bidel, L. P. R., Cazals, G., Ferrare, K., Leroy, J., Faucanié, M., Chevassus, H., Tournier, M., Lajoix, A.-D., Azay-Milhau, J., 2014. Chemical analysis and antihyperglycemic activity of an original extract from Burdock root (*Arctium lappa*). J. Agric. Food Chem. 62, 7738-7745.

Tousch, D., Lajoix, A.-D., Hosy, E., Azay-Milhau, J., Ferrare, K., Jahannault, C., Cros, G., Petit, P., 2008. Chicoric acid, a new compound able to enhance insulin release and glucose uptake. Biochem. Biophys. Res. Commun. 377, 131-135.

Urgate, M., Brown, M., Hollywood, K.A., Cooper, G.J., Bishop, P.N., Dunn, W.B., 2012. Metabolomic analysis of rat serum in streptozotocin-induced diabetes and after treatment with oral triethylenetetramine (TETA). Genome Med. 4, 35. <https://doi.org/10.1186/gm334>.

Villano, D., Fernandez-Pachon, M.S., Moya, M.L., Troncoso, A.M., Garcia-Parrilla, M.C., 2007. Radical scavenging ability of polyphenolic compounds towards DPPH free radical. Talanta. 71, 230-235.

Whiting, D.R., Guariguata, I., Weil, C., Shaw, J., 2011. IDF diabetes atlas: global estimates of the prevalence of diabetes for 2011 and 2030. Diabetes Res. Clin. Pract. 94, 311-321.

Zhen, Y. N., Liu, K.Y., Jia, G.Y., Li, H.P., Han, L.K.; Mucun, S.X., 2007. Effect of hot-water extract of coffee seeds on postprandial blood glucose concentration in rats. Chinese Pharmaceutical journal. 42, 32-35.

Comment citer ce document :

Ferrare, K., Bidel, L., Awwad, Poucheret, Cazals, G., Lazennec, Azay-Milhau, J., Tournier, Lajoix, Tousch, D. (Auteur de correspondance) (2018). Increase in insulin sensitivity by the association of chicoric acid and chlorogenic acid contained in a natural chicoric acid extract (NCRAE) of chicory (*Cichorium intybus* L.) for an antidiabetic effect. Journal of Ethnopharmacology.

Legends of Figures and Tables:

Figure 1: Time course of body weight (A), food intake (B) and Glycosuria (C) in STZ diabetic rats before and during the daily intraperitoneal administrations of NCRAE (15 mg.Kg⁻¹) or SCCAM (15 mg.Kg⁻¹). Control animals received only IP saline solution. Four animals were used for each experimental group and the values are the means (\pm SEM). (**)
p<0.01.

Figure 2: Time course of hyperglycemia (mg.dL⁻¹) in STZ diabetic rats during the daily intraperitoneal administrations of NCRAE (15 mg.Kg⁻¹) or SCCAM (15 mg.Kg⁻¹). Control animals received only IP saline solution. Four animals were used for each experimental group. Basal values were respectively 600 \pm 25 mg.dL⁻¹, 563 \pm 32 mg.dL⁻¹ and 525 \pm 38 mg.dL⁻¹ for control, SCCAM and NCRAE treated diabetic rats. In the box (surrounded by a black border) the glycemia is presented in % from basal values. Values are the means (\pm SEM) by group. (*) p<0.05 and (**) p<0.01.

Figure 3: Effects of daily intraperitoneal administrations of NCRAE (15 mg.Kg⁻¹) or SCCAM (15 mg.Kg⁻¹) on oral glucose tolerance test (OGTT) (glucose at 3g.Kg⁻¹) in STZ diabetic rats. OGTT were performed the 7th day of treatments (n=4 rats for each experimental group) and values are the means (\pm SEM). (**) p<0.01 and (***) p<0.001.

Figure 4: Effect of a SCCAM treatment in comparison of NCRAE on the glucose-uptake in L6 muscle cell line (A) . Values are the means (\pm SD) of three independent experiments. (*) p< 0.05 and (**) p<0.01. Effect of a SCCAM treatment in comparison with NCRAE on the mortality induced by H₂O₂ oxidative stress applied on L6 muscle cell line (B). The duration of the treatment has been of 14 hours before the H₂O₂ application at 40 μ M. Values are the means (\pm SD) of three independent experiments. (*) p< 0.05.

Table 1: LC–MS Fingerprint with Fragmentation of NCRAE Compounds. Peaks obtained are listed in the order of elution, with their names, molecular formulas, precursor ions, and fragmentation data. TR (min): retention time in minutes. Mol. form.: molecular formula. Theo. mass: theoretical monoisotopic mass of precursor ion $[M - H]^-$. Δ ppm: mass tolerance expressed in parts per million.

peak	RT (min) ^b	Parent ion mol. form. ^c	Parent ion theoretical mass (m/z) ^d	Fragmentation data (m/z) $[M-H]^-$
P1	10.21	C ₁₃ H ₁₂ O ₉	311.0403	311(100), 179(89), 149(100), 135(32)
P2	12.24	C ₁₃ H ₁₂ O ₉	311.0403	311(100), 179(40), 149(25), 135(4)
P3	19.83	C ₁₆ H ₁₈ O ₉	353.0878	179(75), 161(10), 135(19), 85(25)
P4	21.64	C ₁₆ H ₁₈ O ₉	353.0878	191(100), 85(12)
P5	22.59	C ₁₆ H ₁₈ O ₉	353.0878	191(34), 179(75), 173(100), 135(26)
P6	23.63	C ₂₂ H ₁₉ O ₁₃	491.0794	329(100), 311(10), 293(80)
P7	35.19	C ₂₂ H ₁₈ O ₁₂	473.0720	311(100), 293(115), 219(10), 179(20), 149(31), 135(44)
P8	37.20	C ₂₂ H ₁₈ O ₁₂	473.0720	311(100), 293(88), 219(6), 179(55), 149(24), 135(17)
P9	38.10	C ₂₂ H ₁₈ O ₁₂	473.0720	311(100), 293(95), 219(5), 179(23), 149(25), 135(30)
P10	39.46	C ₂₁ H ₁₈ O ₁₃	477.0669	301(100), 273(5), 245(10), 151(120), 179(45)
P11	40.39	C ₂₁ H ₁₈ O ₁₂	461.0720	285(100), 93(12)
P12	40.86	C ₂₅ H ₂₄ O ₁₂	515.1189	353(100), 335(10), 191(7), 179(19), 173(25)
P13	41.80	C ₂₅ H ₂₄ O ₁₂	515.1195	353(100), 191(12), 179(4), 85(10)
P14	43.47	C ₂₅ H ₂₄ O ₁₂	515.1195	353(100), 191(15), 179(7), 149(12), 135(18)
P15	43.6	C ₂₁ H ₁₈ O ₁₂	461.0720	285(100), 93(10)
P16	44.36	C ₂₅ H ₂₄ O ₁₂	515.1195	353(100), 299(8), 203(14), 191(10), 173(25), 179(10)
P17	47.44	C ₂₃ H ₂₂ O ₁₃	505.0994	463(60), 301(45), 151(22)
P18	50.67	C ₂₇ H ₃₀ O ₁₅	593.1511	447(65), 285(43),
P19	51.02	C ₂₉ H ₂₈ O ₁₅	615.1355	515(15), 453(25), 353(100), 335(5), 191(25),

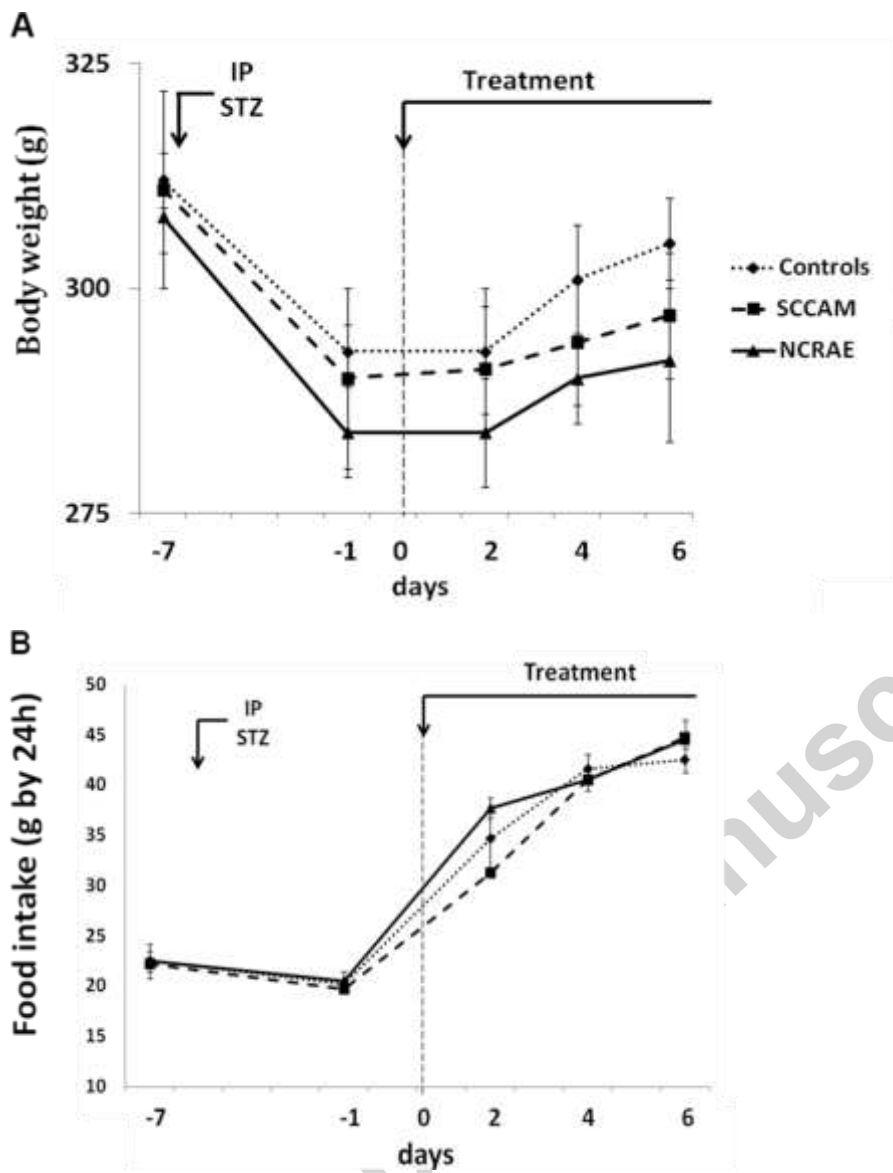
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P20	51.88	C ₂₇ H ₂₈ O ₁₂	543.1468	381(100), 161(37), 149(51), 134(5)
P21	53.51	C ₂₇ H ₂₈ O ₁₂	543.1468	381(100), 161(18), 149(34), 133(4)
P22	53.98	C ₂₄ H ₂₁ O ₁₂	339.0722	177(100), 133(15)
P23	53.98	C ₂₄ H ₂₁ O ₁₂	501.1015	311(100), 293(93), 161(99)

Table 2 : The concentrations of each hydroxycinnamic acid were calculated by integrating Peak areas of chromatograms at 326 nm (maximal absorbance of CGA) and are expressed as Equivalent Absorbance of the authentic L-chicoric acid standard at 10⁻³ M.

Peak	RT (min)	Abs ₃₂₆	name
P1	10.21	0.003	<i>cis</i> -O-caffeoyltartaric acid
P2	12.24	8.249	<i>trans</i> -O-caffeoyltartaric acid
P3	19.83	0.294	<i>trans</i> -3-O-caffeoylquinic acid
P4	21.64	0.042	<i>trans</i> -5-O-caffeoylquinic acid
P5	22.59	0.035	<i>trans</i> -4-O-caffeoylquinic acid
P6	23.63	0.179	caffeoyl-dihydroxy-phenyllactoyltartaric acid
P7	35.19	47.853	(2S, 3S)-di-O-caffeoyltartaric acid
P8	37.20	3.955	(2R, 3R)-di-O-caffeoyltartaric acid
P9	38.10	12.407	<i>meso</i> -di-O-caffeoyltartaric acid
P10	39.46	6.630	quercetin-3-O-glucuronide
P11	40.39	0.019	kaempferol-3-O-glucuronide
P12	40.86	1.203	<i>trans</i> -3,4-di-O-caffeoylquinic acid
P13	41.80	7.790	<i>trans</i> -3,5-di-O-caffeoylquinic acid
P14	43.47	3.743	<i>cis</i> -3,5-di-O-caffeoylquinic acid
P15	43.6	0.0001	kaempferol-glucuronide isomer
P16	44.36	5.162	<i>trans</i> -4,5-di-O-caffeoylquinic acid
P17	47.44	0.0001	quercetin-7-O-(6"-acetyl-glucoside)
P18	50.67	0.0001	kaempferol-rhamnosyl-hexoside
P19	51.02	0.0001	1,5-di-O-caffeoyl-3-O-succinoylquinic acid
P20	51.88	0.088	dimethoxycinnamoyl caffeoylquinic acid isomer
P21	53.51	1.261	dimethoxycinnamoyl caffeoylquinic acid isomer
P22	53.98	0.0001	esculin = cichoriin
P23	55.12	1.087	feruloyl-tartaric acid isomer



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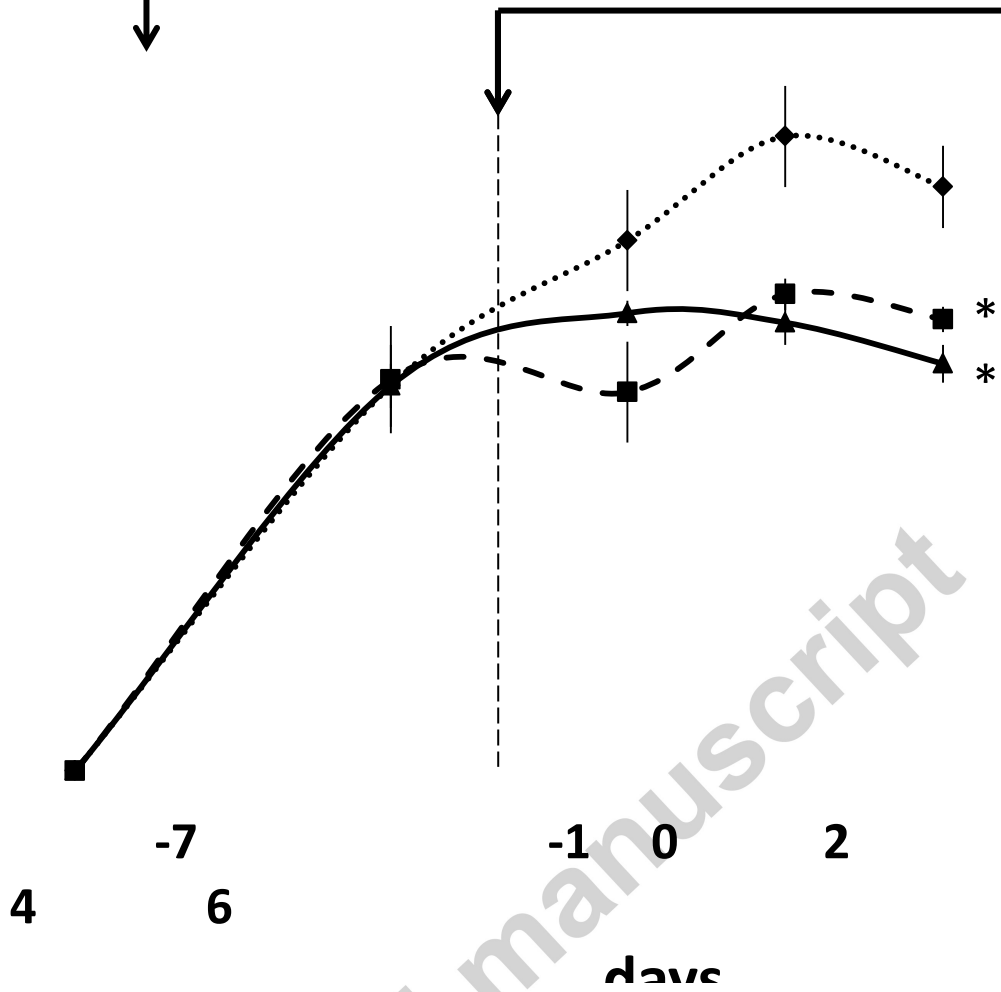


Figure 1

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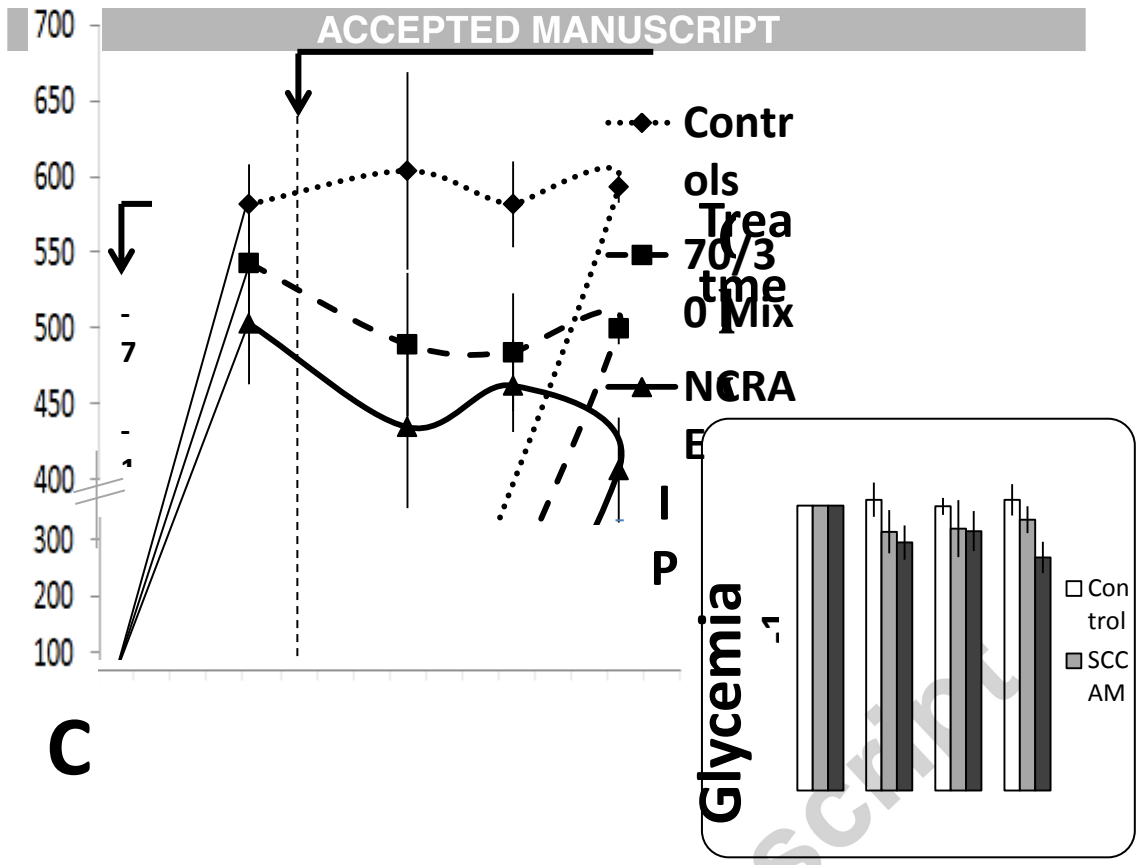


Figure 2

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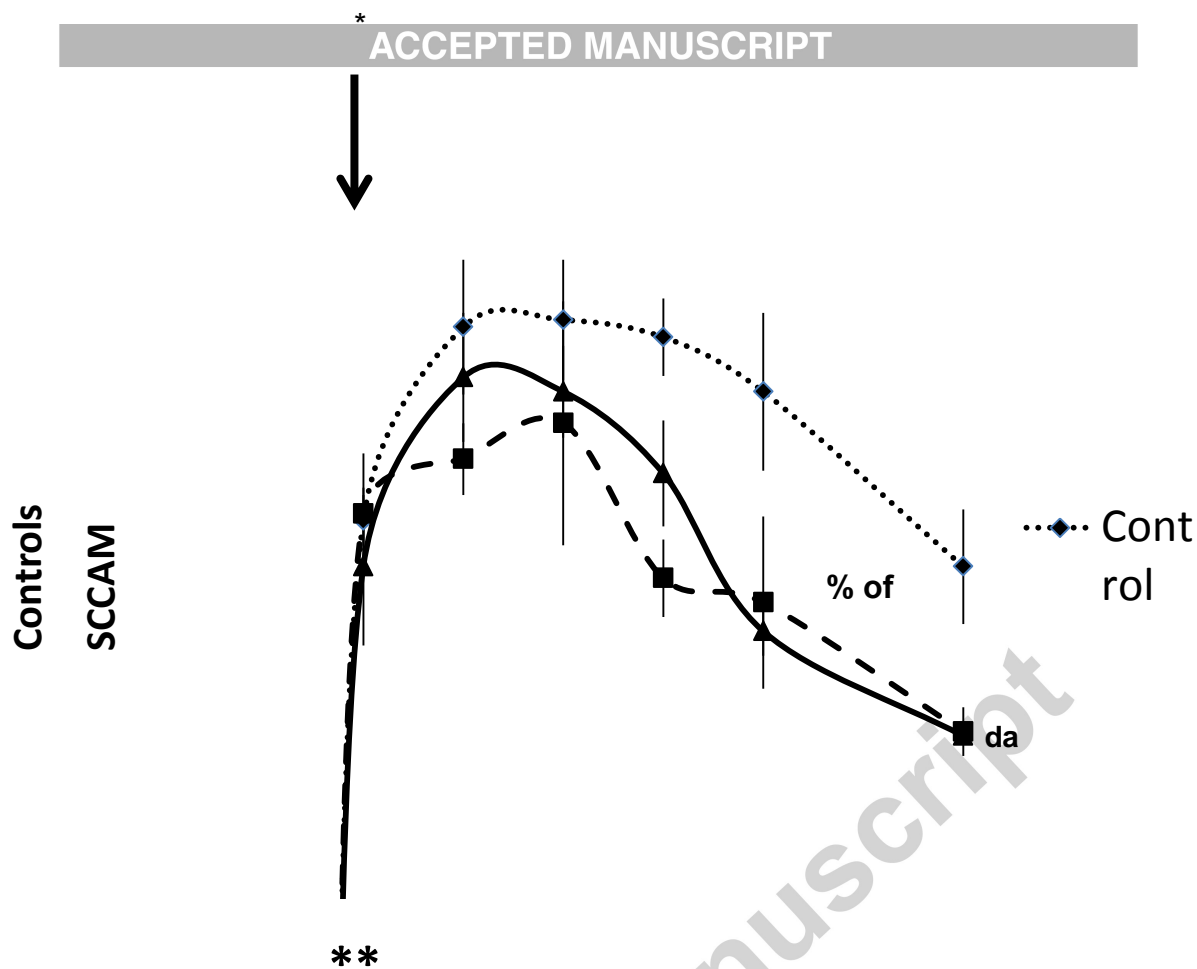
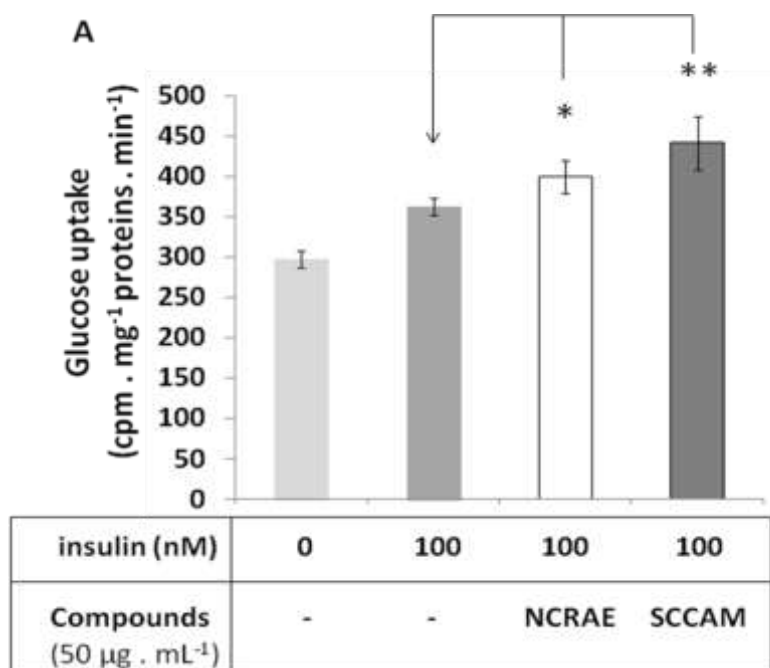


Figure 3

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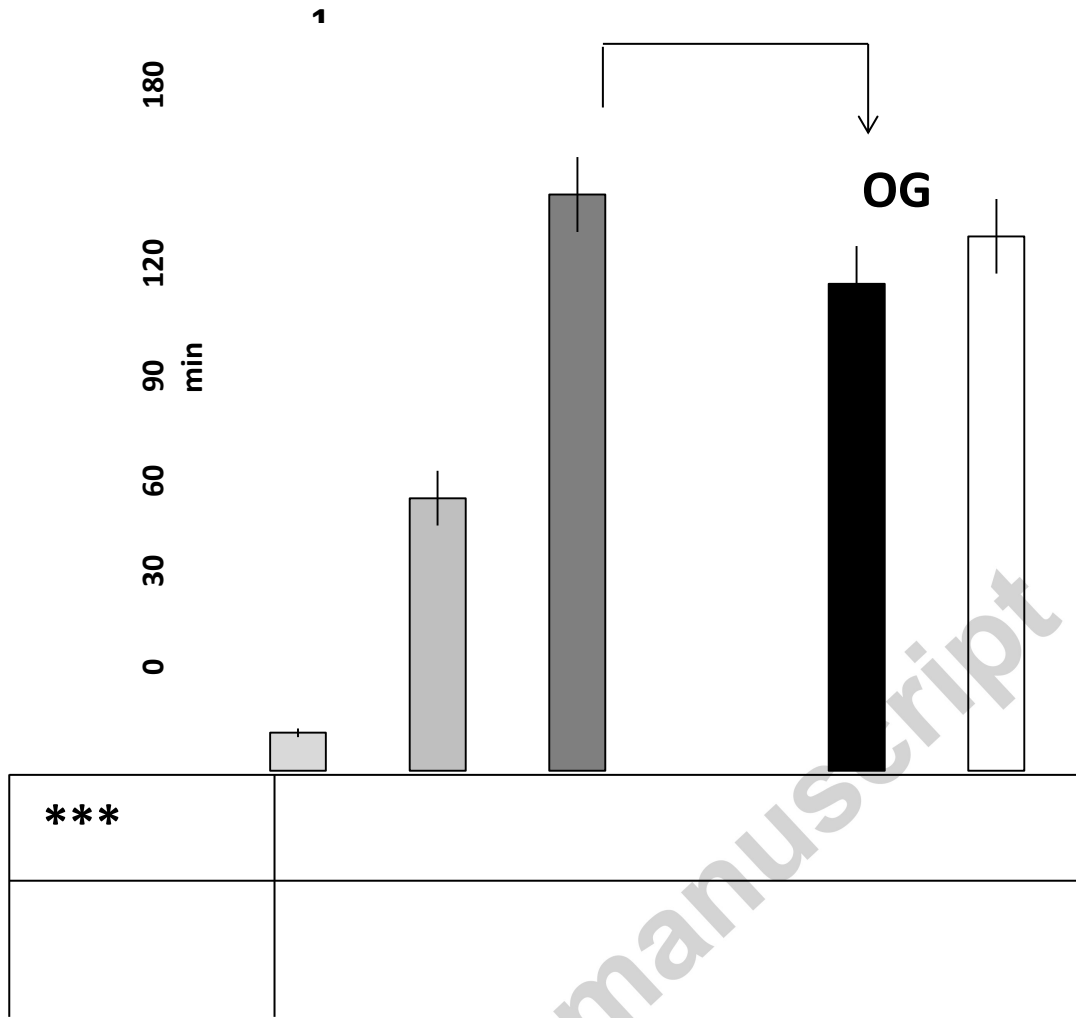


Figure 4

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