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Short-term effect of an aqueous *Fraxinus excelsior* L. seed extract in spontaneously hypertensive rats

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ABSTRACT

We evaluate the short-term effect of a *Fraxinus excelsior* L. seed extract, named FraxiPure (FRP) on spontaneously hypertensive rats (SHR). Water, Captopril and different doses of FRP were orally administered. Blood pressure was recorded by the tail cuff method and plasma samples were collected to determine antioxidant capacity and malondialdehyde equivalents. In other trials, 18 h fasted SHR were administered water, FRP, Furosemide, Torasemide, and Captopril. In these animals, urine was collected for 4 h to obtain the urine volumetric excretion (UVE). Plasma samples of these animals were used to establish the fractional excretion (FE) of different ions (Na^+, Cl^-, K^+, Ca^{2+} and PO_4^{3-}) and metabolites (creatinine, urea and uric acid).
FRP shows antihypertensive effect. The decrease in blood pressure caused by FRP was slightly lower than that of Captopril and more accentuated than that of Torasemide. FRP and Torasemide significantly increased UVE. Torasemide significantly increased FE(Na^+) and FE(Cl^-) and showed a clear uricosuric effect. FRP showed slight natriuretic effects and behaved as a potassium-sparing diuretic. FRP significantly increased plasma antioxidant capacity and decreased plasma malondialdehyde equivalents. The antihypertensive effect of FRP in SHR can therefore be related with its diuretic and antioxidant properties.

1. Introduction

Hypertension is considered a very important disease in our society given its high prevalence and its repercussion in other different cardiovascular disorders. Even if the diagnostic and pharmacological tools make nowadays easy to treat this pathology, only few hypertensive patients maintain adequate arterial blood pressure levels. Moreover, the patients with a pharmacological control of this variable frequently bear unpleasant side effects. In recent years, society has become increasingly aware of the close relationship that exists between diet and health, and, in particular, between the consumption of foods rich in antioxidants and the drop of arterial blood pressure.

Fraxinus excelsior L. is widely distributed throughout the southeastern region of Morocco (Tafilatet) where is locally known as "l´assane l´ousfour". This deciduous shrub, that belongs to the *Oleaceae* family, is known a long time ago worldwide in phytomedicine as an antioxidant (Meyer, Schneider, & Elstner, 1995; Schempp, Weiser, & Elstner, 2000), antiinflammatory (El-Ghazaly, Khayyal, Okpanyi, & Arens-Corell, 1992; Von Kruedener, Schneider, & Elstner, 1996), antirheumatic (Von Kruedener, Schneider, & Elstner, 1995), analgesic and antipyretic (Okpanyi, Schirpke-von Paczensky, & Dickson, 1989) plant.

Maghrani et al. (2004) reported that a *F. excelsior* L. seed extract possesses hypoglycemic activity in normal and streptozotocin-induced diabetic rats. Afterwards, oral administration of 20 mg/kg/day of this extract for three weeks produced a significant decrease in the arterial blood pressure of both, spontaneously hypertensive rats (SHR) and normotensive Wistar–Kyoto rats, and this effect was mainly attributed to the diuretic and natriuretic properties of the assayed extract (Eddouks, Maghrani, Zeggwagh, Haloui, & Michel, 2005).

A *F. excelsior* L. seed extract, named FraxiPure (FRP), was obtained by an industrial process (Visen et al., 2009) different from the method used by Eddouks et al. (2005). FRP was previously characterized, being the main constituents the secoiridoid glycoside nuzhenide and the dimeric secoiridoid glycoside GI3 (Visen et al., 2009). Recently, other phenolic compounds have been also identified in FRP (Bai et al., 2010; Ibarra et al., 2011). This extract has also demonstrated beneficial properties on health. FRP lowered the incremental postprandial plasma glucose concentration in non diabetic volunteers (Visen et al., 2009) and limited weight gain and hyperglycemia in high-fat diet-induced obese mice (Ibarra et al., 2011). Nevertheless, the eventual antihypertensive activity of this extract has not been still tested. Therefore, the aim of this study was to evaluate the short-term effect of FRP on spontaneously hypertensive rats (SHR), and to investigate the possible diuretic and antioxidant properties of this extract in these animals.

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2. Material and methods

2.1. F. excelsior L. seed extract, animals and general conditions

The extract used in this study (FRP) was prepared as described by Visen et al. (2009). Briefly, the seeds were milled, and then the seed powder was extracted in water stirring for 2 h at 65 °C. After filtration, the solution was concentrated under vacuum at 40 °C which was then mixed with carriers and spray-dried to obtain a fine powder. This is the preferred method of drying of many thermally-sensitive materials such as foods that takes a liquid stream and separates the solute as a solid and the solvent into a vapor.

In this study, we have used 17–20-week-old male SHR, weighing 310–320 g, and 17–20-week-old male normotensive Wistar–Kyoto (WKY) rats, weighing 330–350 g. All these animals were obtained from Charles River Laboratories Spain. The rats were maintained at a temperature of 23 °C with 12 h light/dark cycles. They consumed tap water and a standard diet (A04 Panlab, Barcelona, Spain) *ad libitum*, and the experiments described below were performed as authorized for scientific research (European Directive 86/609/CEE and RD 223/1988 of the Spanish Ministry of Agriculture, Fisheries and Food).

2.2. Experimental design to evaluate arterial blood pressure effect

Different doses (5, 10, 20, 30 and 40 mg/kg) of FRP were administered by gastric intubation to the SHR between 9 and 10 am. Distilled water was used as negative control, and Captopril (Sigma, USA) (50 mg/kg), a known antihypertensive drug, was given as positive control to the SHR. 20 mg/kg FRP was also evaluated in the WKY and compared with the negative control in these animals. In these trials, and also in the trials described afterwards, the volume orally administered to the rats was always 1 ml/rat either of water, or of the appropriate water solution.

Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were recorded in the rats by the tail cuff method before administration and 2, 4, 6, 8, 24 and 48 h post-administration. In addition in order to evaluate the possible antihypertensive effect of Furosemide and Torasemide in our study, 10 mg/kg Furosemide and 1 mg/kg Torasemide were also respectively administered to the SHR by the same procedure by the mentioned above. Arterial blood pressure was measured in these animals just before administration and 4 h post-administration.

Before the measurement of arterial blood pressure, the rats were kept at 38 °C for 10 min in order to detect the pulsations of the tail artery. The original method for measuring arterial blood pressure using the tail cuff provides only SBP values (Buñag, 1973), but the equipment used in this study, LE 50001 (Letica, Hospitalet, Barcelona, Spain), has a high sensitivity pulse transducer coupled with an accurate microprocessor program, and allows us to distinguish between SBP and DBP. To establish the value of SBP and DBP, five measurements were taken, and the average of all of them was obtained. To minimize stress-induced variations in blood pressure all measurements were taken by the same person in the same peaceful environment. Moreover, to guarantee the reliability of the measurements we established a training period of two weeks before the actual trial time, and during this period the rats were accustomed to the procedure.

2.3. Experimental design to evaluate the diuretic effect

The diuretic effect of FRP was evaluated following the method of Lipschitz, Haddian, and Kerpscar (1943) modified by Ramírez et al. (Lipschitz et al., 1943; Ramírez, Palacios, & Gutiérrez, 2006). SHR were fasted for 18 h and administered by gastric intubation the following products: water (1 ml), 20 mg/kg FRP, 50 mg/kg Captopril, 10 mg/kg Furosemide and 1 mg/kg Torasemide. Immediately after these administrations, the rats were individually housed in metabolic cages and urine was collected for 4 h in order to obtain the urine volumetric excretion (UVE). This parameter was calculated by the following formula: volume urine collected × 100 / volume of liquid intake. Urine samples were stored at −80 °C. Blood samples were obtained in the animals from the different groups just after collecting the urine. The blood samples were centrifuged and the corresponding plasma samples were stored at −80 °C.

The concentration of different ions (Na^+, Cl^-, K^+, Ca^{2+} and PO_4^{3-}) and metabolites (creatinine, urea and uric acid) in the urine samples were obtained and the same quantitative determinations were carried out in plasma samples as described below. Creatinine clearance (Ccr) was calculated and we obtained also the fractional excretion (FE) of the ions and products mentioned above. FE was calculated according to the following formula: 100 × [urine product] × [plasma creatinine] / [plasma product] x [urine creatinine]. This parameter reflects the percentage of the ion or product filtered by the kidney which is excreted in the urine.

A Cobas Integra 400 analyzer was used for urine determinations and a Roche modular D 2400 apparatus (Barcelona, Spain) was used for plasma determinations. All the products used for these determinations were obtained from Roche Diagnostics (Barcelona, Spain). We used ion selective electrodes for the quantitative determination of Cl^-, Na^+, and K^+. The concentration of inorganic phosphate was determined by a photometric method by measuring the increase in absorbance at 340 nm of the ammonium phosphomolybdate complex that was formed when the original product reacts with ammonium molybdate in the presence of sulphuric acid (phosphomolybdate method). The concentration of calcium was also determined by a photometric method. In the urine samples we measured the increase in absorbance at 552 nm of the ethylenediaminetetraacetic acid (EDTA)–calcium complex that was formed when Ca^{2+} reacts first with 5-nitro-5′-metil-1, 2-bis(o-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid) (NM-BAPTA) and immediately after with EDTA, and in plasma samples we used the calcium-o-cresolphtalein complexone method. The concentration of uric acid was determined by the uricase (urate oxidase that catalyzes the oxidation of uric acid to allantoin) method. The concentration of the quinone diimine that was formed in the final reaction by applying this enzymatic colorimetric method was estimated by the increase in absorbance of the corresponding solution at 552 nm. To determine the urea concentration we added first urease (an enzyme that catalyzes the hydrolysis of urea, forming ammonia and carbon dioxide) and next glutamate dehydrogenase to the urine samples. The absorbance decrease in the final solution was measured at 340 nm. Finally, an espectrophotometrical measurement of creatinine was carried out at 520 nm by obtaining the corresponding picrate complex (Janovski complex) that was formed when the original product reacts with picric acid in an alkaline medium, according to the conventional red Jaffe's reaction (Jaffe, 1886).

2.4. Experimental design to evaluate the antioxidant effect

Plasma samples were collected from SHR administered by gastric intubation water, 20 mg/kg FRP or 50 mg/kg Captopril in order to determine plasma antioxidant capacity and plasma malondialdehyde (MDA) equivalents. To measure the plasma antioxidant capacity in these samples the oxygen radical absorbance capacity assay (ORAC) was used, with some modifications previously described (Manso et al., 2008). MDA levels were measured in these plasma samples by a thiobarbituric acid assay as previously described also by Manso et al. (2008).

2.5. Statistical analysis

The results are expressed as mean values ± standard error of the mean (SEM) and were analyzed by a one-way or two-way analysis of variance (ANOVA), using the GraphPad Prism software. Differences