



## Acute effects of *Fraxinus excelsior* L. seed extract on postprandial glycemia and insulin secretion on healthy volunteers

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### ABSTRACT

**Aim of the study:** *Fraxinus excelsior* L. (Family: Oleaceae) seeds are consumed as a food, condiment, and folk medicine. The seeds are traditionally used as a potent hypoglycemic agent, but no clinical evidence exists in as to this regard. We assessed the clinical efficacy and safety of the seed extract (FraxiPure™, Naturex), containing 6.8% of nuzhenide and 5.8% of GI3 (w/w), on plasma glucose and insulin levels against glucose (50 g) induced postprandial glycemia.

**Materials and methods:** Preselected dose (1.0 g) was used in a double blind, randomized, crossover, placebo (wheat bran) controlled study on 16 healthy volunteers. Each treatment was given immediately after a fasting blood glucose sample (0 min). Postprandial plasma glucose levels were estimated at 0, 15, 30, 45, 60, 90 and 120 min; and postprandial plasma insulin at 0, 30, 60, 90 and 120 min.

**Results:** The extract lowered the incremental postprandial plasma glucose concentration as compared to placebo at 45 min ( $P=0.06$ ) and 120 min ( $P=0.07$ ). It statistically ( $P=0.02$ ) reduced the glycemic area under the blood glucose curve. The seed, also, induced a significant ( $P=0.002$ ) secretion of insulin at 90 min after glucose administration. However, the insulinemic area under the blood insulin curve was not different than the placebo. No adverse events were reported.

**Conclusions:** Our findings confirm the hypoglycemic action of *Fraxinus excelsior* L. seed extract. These promising results, thus, encourage conducting long-term clinical studies to further evaluate the efficacy and safety of *Fraxinus excelsior* L. seed extract in healthy and diabetic volunteers and also to explore the possible mechanism(s) of action.

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### 1. Introduction

Plant derivatives with purported hypoglycemic properties have been used in folk medicine and traditional healing systems around the world (e.g., Native American, Indian, Jewish, Chinese, East Indian, Mexican) (Ungar, 1957; Yeh et al., 2003). But on the other hand, as indicated by Marles and Farnsworth (1995), not all of the plants, based on anecdotal use, reported to be entirely safe, and they emphasize the need for carefully planned scientific research to identify those hypoglycemic plants with true therapeutic efficacy and safety. To provide evidence-based herbal medicine, we must standardize suitable clinical models and utilize randomized controlled trials (RCTs) to determine what herbs are efficacious

for what diseases and standardized them according to their health use.

In this pursuit, the present study, utilizing our well-standardized acute clinical model, was undertaken with the common ash, *Fraxinus excelsior* L. (Family: Oleaceae). It grows naturally in Europe, North Africa, and Asia from the shores of the Atlantic Ocean in the West to the Volga River in the East (Pliûna and Heuertz, 2003; Eddouks et al., 2005). Several reports reveal that the seeds of *Fraxinus excelsior* L. have been traditionally used as food, condiment, and folk medicine (Hedrick, 1919; Kunkel, 1984; Boisvert, 2003). The ash tree is known in Morocco as “Lissan Ettir”, and its seeds as “L'ssane l'ousfour”, one of the ingredients of the condiment “Ras el Hanout” used to prepare the famous tagines and other typical Moroccan plates (Sinclair, 1998; Vergne, 2001; Allen, 2007). In The Netherlands, there is evidence of the use of ash seeds since the medieval 16th century (Vermeeren and Gumbert, 2008). In Iran, the ash seeds are employed as carminative and to destroy

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bladder stones (Parsa, 1959). Also, in Morocco the aqueous extract of the ash seeds is drunk in order to enhance several health conditions (Eddouks and Maghrani, 2004). This plant has been reported to have anti-oxidative (Meyer et al., 1995; Schempp et al., 2000; Middleton et al., 2005), anti-inflammatory (El-Ghazaly et al., 1992; von Kruedener et al., 1996), anti-rheumatic (von Kruedener et al., 1995; Gundermann and Müller, 2007), analgesic (Okpanyi et al., 1989), and antipyretic (Strehl et al., 1995) properties. The seeds of *Fraxinus excelsior* L. were recognized as potent hypoglycemic agents by several traditional healers using it for type 1 and type 2 diabetes mellitus (Eddouks et al., 2005). Eddouks and Maghrani (2004) and Maghrani et al. (2004) experimented on animals and reported hypoglycemic activity in normal rats and anti-diabetic properties, such as controlling streptozotocin (STZ) induced hyperglycemia, but no clinical evidence and scientific validation exist in this regard.

Therefore, in the current acute clinical study, for the first time an attempt has been made to confirm our hypothesis that *Fraxinus excelsior* L. seed extract will reduce postprandial glycemia in non-diabetic healthy individuals following glucose (50 g) intake. In order to determine the underlying mechanism, the serum insulin concentrations were also determined. We are thus; presenting the preliminary data on the acute clinical effect of the aqueous extract of the seeds of *Fraxinus excelsior* L. on healthy subjects against glucose induced postprandial glycemia.

## 2. Materials and methods

### 2.1. *Fraxinus excelsior* L. seed extract

#### 2.1.1. Raw material

The seeds of *Fraxinus excelsior* L. were collected from many rural communities in Morocco and deposited at the herbarium (voucher specimen # J02/02/A7; reference # RB3524) of Naturex Maroc, Technopole Nouasser BP 42, Casablanca 20240, Morocco. First the samaras were harvested from the trees in the Atlas Mountains, and then the seeds were separated manually at home, a traditional practice in that region. After the collection, the seeds were analyzed in order to confirm their botanical origin. Analyses included macroscopic, microscopic and high pressure thin layer chromatography (HPTLC, CAMAG, Switzerland) techniques. These analyses were conducted by Mr. Elan Sudberg from Alkemists Pharmaceuticals, Inc. (Costa Mesa, CA, USA) using authenticated *Fraxinus excelsior* L. seeds as a control. The sample used in our experiment corresponded to the seeds of *Fraxinus excelsior* L.

#### 2.1.2. Extract preparation

*Fraxinus excelsior* L. seed extract was obtained by an industrial process (FraxiPure™, batch # 347/53/A7; reference # 149251, Naturex SA, Site d'Agroparc BP 1218, 84911 Avignon Cedex 9, France) according to the traditional method used in Morocco (Eddouks and Maghrani, 2004; Maghrani et al., 2004; Eddouks et al., 2005). First the seeds were milled, and then the seed powder was extracted in water stirring for 2 h at 65 °C. The ratio (*Fraxinus excelsior* L. seeds: solvent) was fixed, using only water as a solvent. After filtration, the clarified solution was concentrated under vacuum at 40 °C which was then mixed with Arabic gum and silicon dioxide as carriers and spray dried to obtain a fine powder. Moisture content in the extract was less than 8%. The extract ratio was approximately 6:1 (*Fraxinus excelsior* L. seeds: extract powder), yielding 16.67% of dry *Fraxinus excelsior* L. seed extract.

#### 2.1.3. Chemical identification

The molecular weight of nuzhenide ( $\beta$ -D-glucopyranoside, 2-(4-hydroxyphenyl)ethyl, 6-[(2S,3E,4S)-3-ethylidene-2-( $\beta$ -D-glucopyranosyloxy)-3,4-dihydro-5-(methoxycarbonyl)-2H-

pyran-4-acetate]) and GI3 (2H-pyran-4-acetic acid, 3-ethylidene-2-( $\beta$ -D-glucopyranosyloxy)-3,4-dihydro-5-(methoxycarbonyl)-, 4-[2-[[6-O-[[3-ethylidene-2-( $\beta$ -D-glucopyranosyloxy)-3,4-dihydro-5-(methoxycarbonyl)-2H-pyran-4-yl]acetyl]- $\beta$ -D-glucopyranosyl]oxy]ethyl]phenyl ester, stereoisomer (9CI)), the main secoiridoids being standardized in *Fraxinus excelsior* L. seed extract, were identified by HPLC–MS and the chemical structures were determined by comparison of NMR data to those in literature (LaLonde et al., 1976). Fig. 1 shows the chromatogram of the extract and the chemical structure of the two compounds. An HPLC method was developed for the quantification of the secoiridoid contents. The HPLC system used was an Agilent 1100 (Palo Alto, CA, USA) equipped with a diode array detector. The stationary phase was a Prodigy ODS3 analytical column (250 × 4.6 mm i.d., 5  $\mu$ m, Phenomenex, Torrance, CA, USA) thermostated at 30 °C. The flow rate was 1 ml/min, and the elution was monitored at 238 nm. The mobile phases were (A) water with 0.1% TFA and (B) acetonitrile. The solution of 80% A and 20% B was maintained for 5 min and then changed to 70% A and 30% B after 15 min total time; followed by a linear gradient of 100% B after 25 min total time, maintaining this composition for 10 min; the system was then reequilibrated to the initial composition after 10 min. Peaks of nuzhenide and GI3 appeared at approximately 13 and 18 min, respectively. The *Fraxinus excelsior* L. seed extract used in this clinical trial contained 6.8% of nuzhenide and 5.8% of GI3.

### 2.2. Clinical trial

#### 2.2.1. Test material

The dose of *Fraxinus excelsior* L. seed extract in our acute clinical study was 1.0 g (powdered extract filled in two capsules of 500 mg each).

#### 2.2.2. Placebo

Wheat bran (Certified Hard Red Wheat Bran; lot 195) served as placebo which was also administered at the dose of 1.0 g for comparison. It is certified by American Association of Cereal Chemists (AACC). The main composition of wheat bran consists of protein – 16.05% (Nx6.31), fat – 4.34% (as triglycerides, total), total dietary fiber – 49.65%, carbohydrates – 65.75%, starch – 13.3% (Modified Ewers method), besides vitamin B<sub>1</sub>, B<sub>6</sub>, B<sub>12</sub>, magnesium, potassium, phosphorus, manganese, calcium, copper, iron, sodium and zinc. It did not seem to improve blood glucose control or risk factors for coronary heart disease (CHD) in type 2 diabetes over 3 months (Jenkins et al., 2002).

### 2.3. Study design

The study was randomized, double blind, placebo-controlled and crossover designed. By this design subjects act as their own controls. Only the “blinder”, an independent clinical research scientist, knew the identity of the treatments who performed and maintained the “blinding” of packages, labels, and randomization of the treatments while not having contact with individuals or data. Randomization was done using a random number table. Monitoring of all individual records, data sheets, sample handling, laboratory sample storage, and sample inventory for completeness and adherence to the protocol was conducted by the study monitor.

The trial was carried out at Kumar Clinic and Diabetes Care Centre, Lucknow, India. All the healthy subjects were recruited at this Centre.

#### 2.3.1. Inclusion criteria

- Subjects were males or non-pregnant females aged 18–55 years, recruited from India.

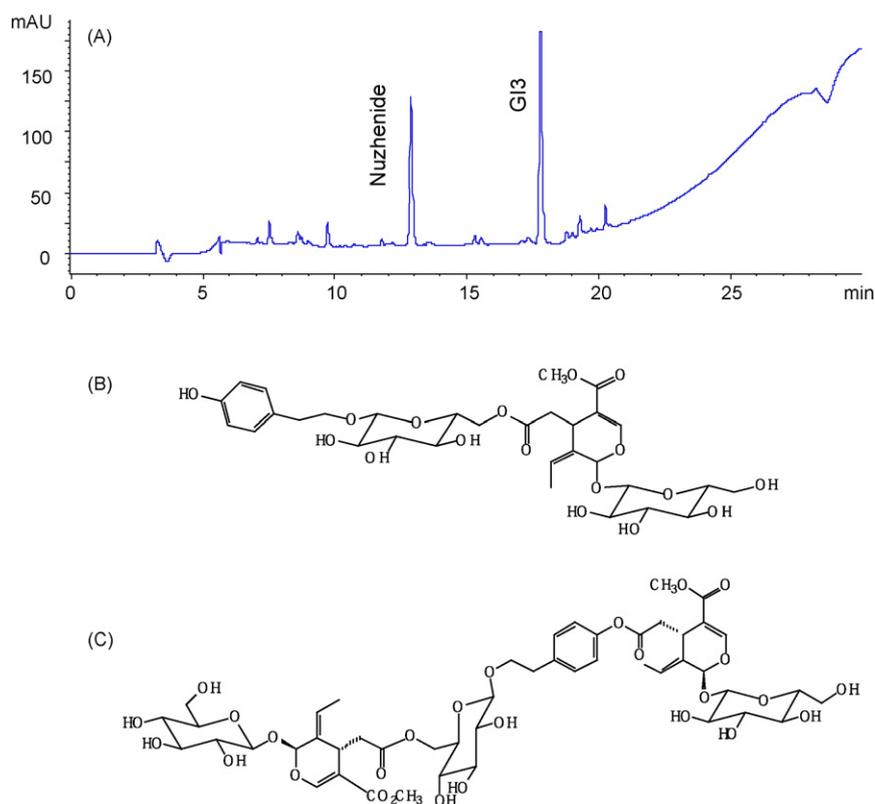


Fig. 1. (A) Chromatogram of *Fraxinus excelsior* L. seed extract, (B) Nuzhenide and (C) GI3.

- Given written consent to participate in the study.
- Healthy, fasting plasma glucose range: 4.0–5.5 mmol/l.
- BMI 25–28 kg/m<sup>2</sup>.

### 2.3.2. Exclusion criteria

- Diabetes.
- Hypertension.
- Any medication which might, in the opinion of investigator, be dangerous to the subject or will affect the results.
- Smokers.
- Heavy alcohol abuse.
- Renal, hepatic or inflammatory bowel disease, anemia or CVD.
- Blood donation within 2 months.
- Subjects who cannot comply with the experimental procedures or do not follow safety guidelines.
- Women with anticipated pregnancy/pregnant/lactation.
- Use of any microsomal enzyme inhibiting/inducing drugs within 30 days and/or systemic medication including OTC operation 14 days prior to day 1 of the study.
- Participation of any clinical trial within 6 weeks preceding day 1 of the study.

### 2.3.3. Participant characteristics and study flow

Prior to commencement of the study, the participants were invited to come to the Kumar Clinic and Diabetic Care Centre, Lucknow (India). They were then briefed with objective and procedures of the study in simple communicable language. All the volunteers were screened for inclusion/exclusion criteria and asked to understand and sign the informed consent form at each visit. During the screening session, they underwent multiple measurements such as blood pressure, height and weight. None of the partici-

pants reported use of natural health products or supplements with potential effects on glycemia with 3 weeks prior to the screening visit.

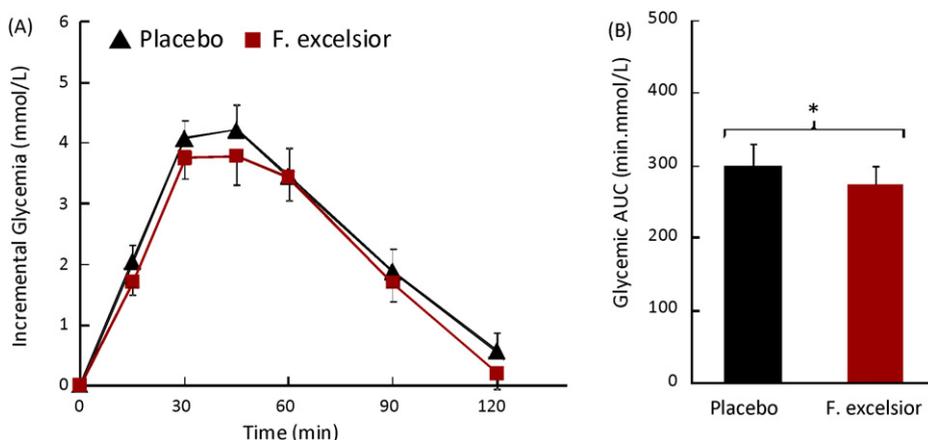
Sixteen healthy individuals (11 male; 5 female of Indian origin, age range: 20–55 years, BMI:  $26 \pm 2.2$  kg/m<sup>2</sup>; fasting blood glucose:  $4.4 \pm 0.09$  mmol/l) completed the study.

The test substance (*Fraxinus excelsior* L. seed extract, 1.0 g) or the placebo (wheat bran, 1.0 g) was administered orally on two separate occasions, with a gap of 1 week (washout period), in the form of two capsules (each having 500 mg) as a single dose prior to the glucose challenge (50 g in 100 ml) for evaluation of glycemic response. The placebo matched with the test substance in all aspects except for active constituents for the purpose of blinding. Each of the 16 healthy subjects underwent randomization for each of the investigated treatment and all of their data were included in final analysis.

At each study visit, 10–12 overnight fasted volunteers first had their blood pressure, weight and height measured and subsequently rested in the seated position. Thereafter, individuals filled out forms detailing their pharmacological regimen including their previous visit and their diet (dinner) and activity (sleep, urination and morning routine) regimen for the previous 12 h.

### 2.3.4. Compliance and symptoms

The participants were asked to maintain their usual carbohydrate intake which excludes dieting or restricting intake and to maintain a constant level of physical activity and lifestyle patterns throughout the course of the study and avoid strenuous exercise the day before and the morning of the test. Moreover, they were advised to refrain from taking any herbal supplements or medicines. Volunteers were asked to have a simple diet 3 days prior to the test substance administration accompanied by 10–12 h of fasting before



**Fig. 2.** Comparison between *Fraxinus excelsior* L. seed extract (1.0 g) and matched wheat bran placebo (1.0 g) on glycemia in healthy volunteers administered with 50 g of glucose. (A) Incremental glycemia at individual time points. (B) Glycemic area under the curve (AUC). Values are mean  $\pm$  SEM. \* $P=0.02$ , Student's  $t$  test ( $n=16$ ).

coming to the clinic between 7:30 and 10:30 a.m. The standardized amount of water and time for treatment consumption were strictly observed by all participants.

### 2.3.5. Methodology

The methods and treatment protocol for the present study have been well established for acute clinical screening at our Risk Factor Modification Centre (RFMC), St. Michael's Hospital, Toronto, Canada (Vuksan et al., 2000, 2001, 2008; Sievenpiper et al., 2003, 2004, 2006).

The consent form, questionnaires along with corresponding protocol were approved by Institutional Human Ethical Committee (IEC) of Kumar Clinic and Diabetes Care Centre vide letter # LNC/FR/2008/19; dated February 15, 2008 following the guidelines of the Declaration of Helsinki and Tokyo for humans and AYUSH, Government of India.

**2.3.5.1. Estimation of blood glucose level.** During the study, finger-prick blood samples were obtained at 0, 15, 30, 45, 60, 90 and 120 min. The test extract/placebo was given immediately with 100 ml of water, after taking out the fasting blood sample at 0 min. This was followed by the ingestion of a glucose (D-glucose; Qualigens Co., Glaxo India) drink (50 g in 100 ml). At this moment the timer was started. This was asked to consume within 5–8 min. Additional finger-prick blood samples were taken at 15, 30, 45, 60, 90 and 120 min after the start of glucose drink. Glucose concentrations were determined in the capillary whole blood using Bayer's glucometer using Essentia glucotrip. The portable blood glucose monitor measures the glucose concentration in whole blood using a glucose dehydrogenase method based on bioamperometry, but is calibrated to yield plasma-like glucose values (Heacock et al., 2005). This blood glucose monitor correlated well with our standard laboratory glucose analyzer (YSI 2300 Stat Plus, Yellow Springs Instruments, Yellow Springs, OH) used routinely at RFMC, St. Michael's Hospital, Canada. During the 2 h of the testing session, subjects remained seated, did not smoke, eat or drink. After the end of study, they were asked to fill up post-test questionnaire. The entire time spent in the clinic was approximately 2.5 h. Thereafter, they were offered a snack and then allowed to leave.

**2.3.5.2. Estimation of serum insulin level.** Venous blood samples (7–8 ml) were collected at 0, 30, 60, 90 and 120 min in test extract/placebo treated healthy subjects in serum separator tubes. The blood was allowed 15 min to clot, and then was centrifuged at  $1500 \times g$  for 10 min. The resulting serum was then analyzed for

insulin using an electrochemiluminescence immunoassay (ECLIA) at the prestigious analytical unit of Ranbaxy at Mumbai, India. The inter-assay CV was between 1.5 and 2.0%.

## 3. Statistical analysis

The positive incremental area under the curve (i-AUC) for both placebo and *Fraxinus excelsior* L. seed extract treated groups were calculated for glycemic and insulinemic concentrations at different time intervals, ignoring areas below the initial value at time zero (Wolever et al., 1991). Significant differences between groups were calculated using a two-tailed paired Student's  $t$  test. Analyses were performed using XLSTAT 2008 software (Addinsoft™, USA). Statistical significance was set at  $P < 0.05$ . All data are reported as mean  $\pm$  SEM.

## 4. Results

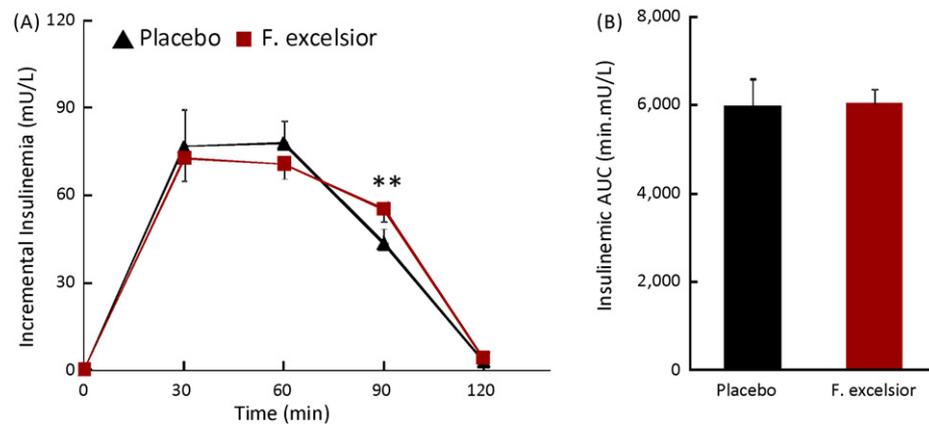
### 4.1. Blood glucose level

The graphic, from pair-wise comparison, of incremental glycemia showed a decrease in postprandial glucose levels by *Fraxinus excelsior* L. seed extract, during the length of experiment from 15 ( $2.0 \pm 0.28$  mmol/l vs.  $1.7 \pm 0.21$  mmol/l), 30 ( $4.1 \pm 0.31$  mmol/l vs.  $3.8 \pm 0.33$  mmol/l), 45 ( $4.2 \pm 0.41$  mmol/l vs.  $3.8 \pm 0.47$  mmol/l), 60 ( $3.5 \pm 0.46$  mmol/l vs.  $3.4 \pm 0.41$ ), 90 ( $1.9 \pm 0.38$  mmol/l vs.  $1.7 \pm 0.31$  mmol/l) to 120 ( $0.58 \pm 0.29$  mmol/l vs.  $0.21 \pm 0.28$  mmol/l) min as compared to matched wheat bran placebo (Fig. 2A). The extract almost reached statistical difference at 45 min ( $P=0.06$ ) and 120 min ( $P=0.07$ ).

Paired Student's  $t$  test indicated that differences ( $299.8 \pm 28.9$  min mmol/l vs.  $273.3 \pm 25.3$  min mmol/l) in the effect of treatment (*Fraxinus excelsior* L. seed extract vs. placebo) on mean glycemic area under the curve (AUC) were statistically significant ( $P=0.02$ ). The results are presented in Fig. 2B.

### 4.2. Insulin level

*Fraxinus excelsior* L. seed extract ( $55.5 \pm 4.6$  mU/l) induced a significant ( $P=0.002$ ) secretion of insulin at 90 min compared to placebo ( $43.5 \pm 5.0$  mU/l) (Fig. 3A). No significant difference was noticed in the mean insulinemic AUC (0–120 min), in *Fraxinus excelsior* L. seed extract treated group ( $6041.6 \pm 340.5$  min mU/l) compared to placebo ( $5996.3 \pm 594.58$  min mU/l) (Fig. 3B).



**Fig. 3.** Comparison between *Fraxinus excelsior* L. seed extract (1.0 g) and matched wheat bran placebo (1.0 g) on insulin levels in healthy volunteers administrated with 50 g of glucose. (A) Incremental insulinemia at individual time points. (B) Insulinemic area under the curve (AUC). Values are mean  $\pm$  SEM. \*\* $P=0.002$ , Student's  $t$  test ( $n=16$ ).

## 5. Discussion

In the present preliminary acute study with *Fraxinus excelsior* L. seeds, we have tested the aqueous extract in healthy subjects against glucose (50 g) induced postprandial hyperglycemia to know its efficacy and safety. A regular decreasing trend of postprandial glycemia was observed with *Fraxinus excelsior* L. seed extract (1.0 g) which almost reached statistical difference at 45 min ( $P=0.06$ ) and 120 min ( $P=0.07$ ) compared to placebo (wheat bran). This finding, thus, validates the hypoglycemic activity in normal animal studies observed by Eddouks and Maghrani (2004), and Maghrani et al. (2004).

The healthy volunteers were taken in order to avoid potential interaction with anti-diabetic and other medications. Further, the acute approach also eliminates possible concerns regarding the untested safety of the extract. In our investigation, no adverse event or reaction was observed in the healthy volunteers at least for the dose and duration used. However, the effect should nevertheless, be confirmed in long-term trial in healthy as well as diabetic patients. The animal studies are also indicative for such clinical study with *Fraxinus excelsior* L. seed extract where more activity was observed in diabetic rats compared to normal animals (Eddouks and Maghrani, 2004).

In this study, we found that the main compounds in *Fraxinus excelsior* L. seed extract are the secoiridoid glycoside nuzhenide (6.8%) and the dimeric secoiridoid glycoside G13 (5.8%) (Fig. 1), which may be responsible for efficacy. Similar secoiridoid glycosides such as oleuropein, ligstroside, and excelsioside and G15 – a molecule similar to G13 – have also been isolated from *Fraxinus excelsior* L. leaves (Jensen and Nielsen, 1976; Damtoft et al., 1992; Egan et al., 2004). All these secoiridoids glycosides have been reported for hypoglycemic (Gonzalez et al., 1992; Al-Azzawie and Alhamdani, 2006) and anti-diabetic (Ahmed et al., 2003; Somova et al., 2003; Yamabe et al., 2007) properties.

The incremental glycemic curve for *Fraxinus excelsior* L. seed extract shows two different phases compared to the placebo (Fig. 2A). The first phase takes place during the initial 60 min of the experiment. During the first 15 min there is a slight reduction of glycemia in the *Fraxinus excelsior* L. seed extract group, this difference is more evident at 30 min, and almost reaches statistical difference at 45 min ( $P=0.06$ ). The two groups have similar glycemic values at 60 min. The second phase takes place during the last hour of the experiment. At 90 min there is a slight reduction in glycemia in the *Fraxinus excelsior* L. seed extract group compared to the placebo, which almost reach statistical difference at 120 min ( $P=0.07$ ). These two phases may underlay different mechanisms of

action of *Fraxinus excelsior* L. seed extract in reducing postprandial glycemia.

A possible explanation for the first phase is that *Fraxinus excelsior* L. seed extract partially inhibits the absorption of glucose by blocking its uptake in the intestine. The partial inhibition of glucose uptake by other secondary metabolites of plants already have been reported for glycoside flavonoids (Johnston et al., 2005; Kottra and Daniel, 2007; Kwon et al., 2007). Flavonoids reduce the intestinal glucose absorption by limited inhibition of sodium-dependent co-transporter SGLT1 in the brush border membrane (Welsch et al., 1989; Kottra and Daniel, 2007) and the sodium-independent glucose transporter GLUT2 in the basolateral membrane (Kwon et al., 2007). In flavonoids, this inhibition is enhanced in the presence of glycosides (Johnston et al., 2005; Kottra and Daniel, 2007). Therefore, the reduction in glycemia during the first phase might be caused by the glycoside secoiridoids present in *Fraxinus excelsior* L. seed extract by blocking the intestinal glucose transporters. However, more research is needed in order to confirm this hypothesis. Other mechanisms can also be involved in the reduction of postprandial glycemia during the first phase and may have a longer effect. The literature describe that some glycoside secoiridoids lowers the hypoglycemic activity by reducing the peripheral uptake of glucose (Gonzalez et al., 1992).

Regarding the second phase, our preliminary mechanistic trial suggests that the postprandial hypoglycemic effect may be due to increased insulin secretion, compensated for the reduced release in the time period. Normal insulin secretion is biphasic, with an early burst of insulin release within the first 10 min, followed by a progressively phase of insulin secretion that persists as long as the hyperglycemic state is present. As the first 60 min of oral glucose challenge is considered to be the representative of the early phase of insulin secretion (Vuksan et al., 2001), our data suggest that *Fraxinus excelsior* L. seed extract may be able to increase this phase, the loss of which is a primary defect in type 2 diabetes. We observed a significant ( $P=0.002$ ) increase in the insulin level at 90 min by *Fraxinus excelsior* L. seed extract at the dose of 1.0 g, after glucose administration compared to the placebo (Fig. 3), from where we saw a decline in postprandial plasma glucose level. Our study, thus, also offers stronger support for post-absorptive effects (60–120 min of the oral glucose challenges) such as enhancement of insulin secretion (Vuksan et al., 2001). This might be caused by the action of the secoiridoids glycosides present in the *Fraxinus excelsior* L. seed extract. Compounds from the secoiridoid family have proven to stimulate glucose induced insulin secretion (Gonzalez et al., 1992; Al-Azzawie and Alhamdani, 2006; Zhang et al., 2006) and improve the insulin resistance (Somova et al., 2003). In order to exert insulin

secretion, compounds found in *Fraxinus excelsior* L. seed extract or its metabolites must be bioavailable. There is no evidence in literature on the bioavailability of nuzhenide or GI3; but some studies suggested that secoiridoids with similar structures can be absorbed by the body (Edgecombe et al., 2000; Singh et al., 2008). For example, an *in vitro* study showed that part of oleuropein, the main secoiridoid glycoside found in olives, is hydrolyzed in gastric conditions and another portion is degraded by the colonic microflora (Corona et al., 2006). The authors reported paracellular absorption and/or an active transport mechanism via SGLT1 (Edgecombe et al., 2000). Resulting metabolites could then be subjected to classic Phase I/II biotransformation before reaching general circulation (Corona et al., 2006). As there is no information on bioavailability of nuzhenide or GI3, we can suppose that their mechanism of absorption is close to the absorption of oleuropein. However, more research is needed in order to determine the type of metabolites, their pharmacokinetic mechanisms and to understand the possible insulin stimulation mechanisms of secoiridoids from *Fraxinus excelsior* L. seeds in humans.

In type 2 diabetes, in early phase, the insulin resistance is overcome by a compensatory response from the pancreatic beta cells and euglycemia is maintained. In our study, the stimulation of insulin secretion at 90 min seems to be a direct action of the plant extract on the pancreatic islet cells (Zhang et al., 2006) which returned to normalcy at the end of study (120 min). This may reduce insulin resistance and improve insulin sensitivity in such cases. Further, since there is no significant difference in mean insulinemic AUC between treatment and placebo, the use of extract is safe with no resultant hyperinsulinemia in the following hours post-treatment. This strongly indicates the possible mechanism of action of *Fraxinus excelsior* L. seed extract in reducing postprandial hyperglycemia.

Our preliminary study shows a decrease in plasma glucose AUC of 9% with *Fraxinus excelsior* L. seed extract compared to the placebo. The paired Student's *t* test indicated that differences ( $299.8 \pm 28.9$  min mmol/l vs.  $273.3 \pm 25.3$  min mmol/l) in the effect of treatment (*Fraxinus excelsior* L. seed extract vs. placebo) on AUC (0–120 min) were statistically significant ( $P=0.02$ ) (Fig. 2B). This observation, in our acute study, has a clinical relevance. We have only studied the effect of *Fraxinus excelsior* L. seed extract on postprandial glycemia in healthy individuals, not in those with diabetes. Therefore, based on the current observations, further research is needed to evaluate the effectiveness of the extract in people with diabetes and safety over the long-term clinical investigations.

## 6. Conclusion

To our knowledge, there are no studies on the effect of *Fraxinus excelsior* L. seed extract on postprandial glycemia either in healthy subjects or diabetic patients. For the first time, through our standardized acute clinical studies, we have, thus reported its preliminary significant effect on glucose induced postprandial hyperglycemia and insulin secretion in 16 healthy subjects. Implications of the present results are promising. Our findings suggest that *Fraxinus excelsior* L. seed extract causes an acute insulinotropic effect in humans after a glucose challenge, and therefore promotes insulin sensitivity. Moreover, as the total postprandial insulinemia is not statistically different as compared to placebo, it may significantly prevent the development of insulin resistance. This evidence encourages continuing the research work to determine its bioavailability and possible hypoglycemic mechanism of action. More research is needed to, further, evaluate the efficacy and safety of *Fraxinus excelsior* L. seed extract in healthy volunteers as well as in people with diabetes over a long-term.

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