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Fraxinus excelsior seed extract FraxiPure™ limits weight gains and hyperglycemia in high-fat diet-induced obese mice

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ABSTRACT

Purpose: The aim of this study was to determine whether a *Fraxinus excelsior* L. seed extract, FraxiPure™ (0.5% in the diet), limits weight gain and hyperglycemia in mice. In a previous report, we identified several secoiridoids in FraxiPure™, some of which activated peroxisome proliferator-activated receptor alpha (PPARα) *in vitro* and inhibited the differentiation of 3T3-L1 preadipocyte cells. In a separate study, FraxiPure™ reduced glycemia in healthy volunteers, following an oral glucose tolerance test. These findings suggest that FraxiPure™ has antiobesity and antihyperglycemia effects.
Materials and methods: FraxiPure™ was tested in mice that were fed a high-fat diet over 16 weeks and compared with low-fat and high-fat diet controls. Weight gain, omental and retroperitoneal fat, fasting blood glucose, and fasting blood insulin were measured.
Results: FraxiPure™ reduced gains in body weight by 32.30% ($p < 0.05$), omental fat by 17.92%, and retroperitoneal fat by 17.78%. FraxiPure™ also lowered fasting blood glucose levels by 76.52% ($p < 0.001$) and plasma insulin levels by 53.43% ($p < 0.05$) after 16 weeks. Moreover, FraxiPure™ lowered liver weight gains by 63.62% ($p < 0.05$) and the incidence of fatty livers by 66.67%.
Conclusions: Our novel results demonstrate the antiobesity effects of chronic administration of an *F. excelsior* seed extract and confirm its ability to regulate glycemia and insulinemia. In addition, this extract, which is rich in secoiridoid glucosides, protects against obesity-related liver steatosis.

Introduction

The common ash (*Fraxinus excelsior* L., Oleaceae) is a tree that grows naturally in temperate regions across Europe and Asia (Pliûra and Heuertz 2003; Eddouks et al. 2005) and exists throughout southeastern Morocco (Eddouks et al. 2002). Several reports have demonstrated that ash seeds have been used traditionally as food and condiments (Hedrick 1919; Kunkel 1984; Sinclair 1998; Vergne 2001; Boisver 2003; Eddouks and Maghrani 2004; Maghrani et al. 2004; Eddouks et al. 2005) and administered to improve several health conditions (Parsa 1959; Eddouks and Maghrani 2004).

In Europe, there is evidence that these seeds have been collected since the Middle Ages (Vermeeren and Gumbert 2008). The aqueous seed extract of the ash tree is recognized as an effective hypoglycemic and antidiabetic agent by traditional healers in Morocco (Eddouks et al. 2005). Moreover, ash seed extract has hypoglycemic and antidiabetic effects in normal and streptozotocin-induced diabetic rats (Eddouks and Maghrani 2004; Maghrani et al. 2004).

In a previous study, we developed a well-standardized extract of *F. excelsior* seeds (FraxiPure™, Naturex Inc.) (Visen et al. 2009), in which a glucose screen (50 g) was used to assess the effects of FraxiPure™ on plasma glucose and insulin levels. The intervention was double-blinded, randomized, crossover design that tested FraxiPure™ (1.0 g) versus matching placebo (1.0 g of wheat bran) in 16 healthy volunteers. FraxiPure™ significantly reduced the glycemic area under the curve (Visen et al. 2009).

In a separate study, we identified (**1**) salidroside, a phenolic compound, and 9 secoiridoid glucosides in FraxiPure™: (**2**) oleoside-11-methylester, (**3**) nuzhenide, (**4**) 1‴-*O*-*β*-D-glucosylformoside, (**5**) excelside B, (**6**) Gl3, (**7**) Gl5, (**8**) excelside A, (**9**) ligstroside, and (**10**) oleoside dimethyl ester (Bai et al. 2010). In an *in vitro* study, we demonstrated that compounds **2**–**9** inhibited adipocyte differentiation in 3T3-L1 cells (Bai et al. 2010). Further, FraxiPure™ (at 1:10,000) and secoiridoids **3**, **6**–**8**, and **10** activated a peroxisome proliferator-activated receptor alpha (PPARα) reporter cell system in the range of 10^{-4} M, comparable with 10^{-8} M WY 14,643, a specific PPARα agonist that has robust hypolipidemic effects (Chou et

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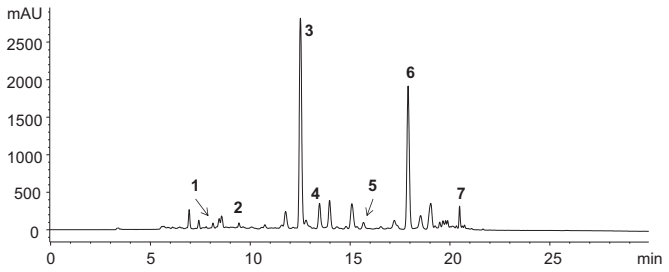


Fig. 1. HPLC chromatogram of *F. excelsior* seed extract (FraxiPure™). The peaks were assigned based on the isolation of each compound, and the structure of the compound was determined by NMR and MS. (**1**) Salidroside, (**2**) Oleoside-11-methylester, (**3**) Nuzhenide, (**4**) 1‴-*O*-*β*-D-Glucosylformoside, (**5**) Excelside B, (**6**) Gl3, and (**7**) Gl5. Peaks of other identified secoiridoid glucosides, present in only trace amounts, are not shown: (**8**) excelside A, (**9**) ligstroside, and (**10**) oleoside dimethyl ester (see Table 1).

al. 2002). This evidence suggests that FraxiPure™ has antiobesity properties.

Based on these findings, the purpose of this study was to examine the antiobesity and antihyperglycemia effects of FraxiPure™ and evaluate its safety in a low-fat/high-fat mouse model.

Materials and methods

F. excelsior seed extract

F. excelsior seeds were collected from rural communities in Morocco and deposited into the herbarium (voucher specimen # J02/02/A7; Reference # RB3524) at Naturex Maroc, Technopole Nouasser BP 42, Casablanca 20240, Morocco. Extracts of the sample were generated through an established industrial process at Naturex France (FraxiPure™, Reference: EA149251, Naturex SA, Site d'Agroparc BP 1218, 84911 Avignon Cedex 9, France) (Visen et al. 2009).

Chromatographic analysis

HPLC analysis was performed on an Agilent 1100 LC Series that was equipped with a quaternary pump, a 4-channel online degasser, an autosampler, a column oven, and a photodiode array detector. Chromatography was performed using a Prodigy ODS3 column (5 μm, 4.6 mm ID × 25 cm) at a flow rate of 1.0 ml/min. The solvent system consisted of 0.1% TFA/H_2O (A) and MeCN (B) as follows: 0–5 min, 0–20% B (v/v); 5–15 min, 20–30% B; and 15–25 min, 30–100% B.

At the end of the run, the column was flushed with 100% MeCN for 10 min, and the column was equilibrated with the starting eluent for an additional 10 min of post-run time. The UV detector was operated at 238 nm, and the column temperature was ambient. Fig. 1 shows the chromatogram, and Table 1 lists the compounds that were identified in FraxiPure™ (Bai et al. 2010).

Animals and diets

Male C57BL/6J mice (aged 5 weeks) were purchased from Jackson Laboratories (Bar Harbor, ME, USA). Fifty mice were randomly assigned to 1 of 3 groups:

- Negative control group on a low-fat diet (LFD) (10% of energy from fat) ($n = 20$);
- Control group on a high-fat diet (HFD) (60% of energy from fat) ($n = 20$);
- High-fat diet, containing 0.5% FraxiPure™ (FED) (60% of energy from fat) ($n = 10$).

Diets of equally sized pellets were prepared by Research Diets Inc. (New Brunswick, NJ, USA). The mice were housed at room temperature on a 12-h light/12-h dark cycle. Animals subsisted on the experimental diets for 16 weeks with free access to their respective chow and water. Average food and fluid intake and body weight were measured. The entire study was performed in accordance with international guidelines regarding animal experiments (Robert et al. 2004).

Tissue harvesting

Mice were food-deprived for 8 h and sacrificed by CO_2 inhalation after 16 weeks of treatment. Whole blood was obtained by cardiac puncture. Liver, omental fat, and retroperitoneal fat were harvested, rinsed, and weighed. Plasma was isolated by centrifugation at 700 × *g* for 15 min. A liver was considered fatty, based on altered coloration; the percentage of fatty livers was recorded in each group. All samples were stored at −80 °C.

Fasting blood glucose

Fasting blood glucose was measured at 0, 5, 8, 10, 12, 14, and 16 weeks of treatment. Food was removed 8 h prior to the blood glucose measurements, and the cage bedding was changed to minimize the interference from coprophagy. Blood was collected from the tail vein, and glucose levels were measured with a One Touch® Ultra® 2 glucose monitor (LifeScan Inc., Milpitas, CA, USA).

Biochemical analysis of plasma samples

Fasting plasma insulin levels and plasma alanine transaminase (ALT) levels were measured at Week 16 of the treatment after sacrifice. Insulin levels were measured by ELISA (Millipore, Billerica, MA, USA), per the manufacturer's protocol. ALT levels were measured spectrophotometrically with a commercial kit (Catachem Inc., Bridgeport, CT, USA).

Statistical analysis

Results are reported as the mean ± standard error to the mean (SEM). Statistical differences between HFD and FED mice compared with LFD animals were determined by one-way ANOVA with Tukey's post hoc test (GraphPad software, San Diego, CA, USA).

Results

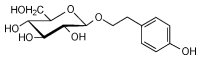
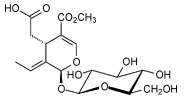
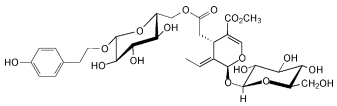
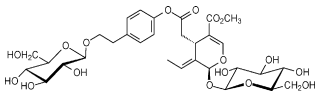
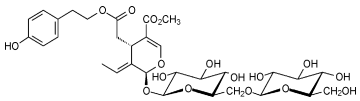
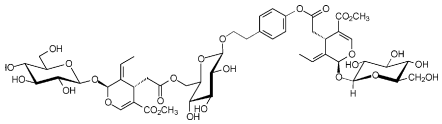
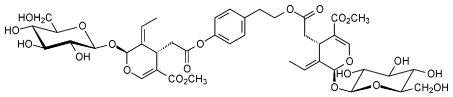
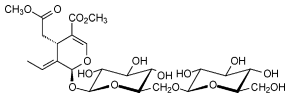
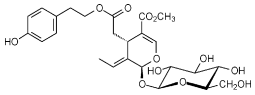
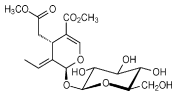
All animals tolerated their respective diets; no adverse effects were observed, the animals behaved normally, and similar average food and water intake was reported for the three groups (data not shown). The FraxiPure™ diet improved physiological and biochemical parameters in mice – i.e., body weight, fat gain in various organs, fasting glucose, and insulin – compared with HFD control animals and the LFD negative control group, the morbid and healthy references, respectively. In addition, FraxiPure™ prevented the development of high-fat diet-induced liver damage.

Changes in body weight, omental fat, and retroperitoneal fat gain

Body weight gain was monitored weekly for 16 weeks in all groups (Fig. 2A). LFD animals grew steadily, plateauing at Week 9 (27.29 ± 0.53 g). In contrast, HFD mice grew regularly throughout the entire study (16 weeks), outpacing LFD animals from Week 3 (25.26 ± 0.49 g *vs.* 23.00 ± 0.42 g – $p < 0.05$) through Week 16 (42.25 ± 1.03 g *vs.* 28.84 ± 0.64 – $p < 0.05$).

Mice that were fed the high-fat diet that contained 0.5% FraxiPure™ (FED) grew similarly to HFD animals until Week 9,

Table 1
Profile of phenolic and secoiridoid compounds in the *F. excelsior* seed extract FraxiPure™.

	Compound	IUPAC name	Structure	Content (%)
1	Salidroside	*β*-D-Glucopyranoside, 2-(4-hydroxyphenyl)ethyl		0.20
2	Oleoside-11-methyl ester	(2*S*,3*E*,4*S*) 2*H*-Pyran-4-acetic acid, 3-ethylidene-2-(*β*-D-glucopyranosyloxy)-3,4-dihydro-5-methoxycarbonyl		0.19
3	Nuzhenide	*β*-D-Glucopyranoside, 2-(4-hydroxyphenyl)ethyl, 6-[(2*S*,3*E*,4*S*)-3-ethylidene-2-(*β*-D-glucopyranosyloxy)-3,4-dihydro-5-(methoxycarbonyl)-2*H*-pyran-4-acetate]		11.42
4	1‴-*O*-*β*-D-Glucosylformoside	(2*S*,3*E*,4*S*) 2*H*-Pyran-4-acetic acid-3-ethylidene-2-(*β*-D-glucopyranosyloxy)-3,4-dihydro-5-(methoxycarbonyl) 4-[2-(*β*-D-glucopyranosyloxy)-ethyl]phenyl ester		1.35
5	Excelside B	(2*S*, 3*E*, 4*S*) 2*H*-Pyran-4-acetic acid-3-ethylidene-2-[(6-*O*-*β*-D-glucopyranosyl-*β*-D-glucopyranosyl) oxy]-3,4-dihydro-5-(methoxycarbonyl) 2-(4-hydroxyphenyl) ethyl ester		0.41
6	Gl3	(2*S*,3*E*,4*S*) 2*H*-Pyran-4-acetic acid, 3-ethylidene-2-(*β*-D-glucopyranosyloxy)-3,4-dihydro-5-(methoxycarbonyl)-, 4-[2-[[6-O-[[3-ethylidene-2-(*β*-D-glucopyranosyloxy)-3,4-dihydro-5-(methoxycarbonyl)- (2*S*,3*E*,4*S*) 2*H*-pyran-4-yl]acetyl]-*β*-D-glucopyranosyl]oxy]ethyl]phenyl ester		6.15
7	Gl5	(2*S*,3*E*,4*S*) 2*H*-Pyran-4-acetic acid, 3-ethylidene-2-(*β*-D-glucopyranosyloxy)-3,4-dihydro-5-(methoxycarbonyl)-, 4-[2-[[[(2*S*,3*E*,4*S*)-3-ethylidene-2-(*β*-D-glucopyranosyloxy)-3,4-dihydro-5-(methoxycarbonyl)-2*H*-pyran-4-yl]acetyl]oxy]ethyl] phenyl ester		0.63
8	Excelside A	(2*S*, 3*E*, 4*S*) 2*H*-Pyran-4-acetic acid-3-ethylidene-2-[(6-*O*-*β*-D-glucopyranosyl-*β*-D-glucopyranosyl) oxy]-3,4-dihydro-5-(methoxycarbonyl) methyl ester		Trace
9	Ligstroside	(2*S*,3*E*,4*S*) 2*H*-Pyran-4-acetic acid-3-ethylidene-2-(*β*-D-glucopyranosyloxy)-3,4-dihydro-5-(methoxycarbonyl) 2-(4-hydroxyphenyl)ethyl ester		Trace
10	Oleoside dimethyl ester	(2*S*,3*E*,4*S*)-2*H*-Pyran-4-acetic acid-3-ethylidene-2-(*β*-D-glucopyranosyloxy)-3,4-dihydro-5-(methoxycarbonyl) methyl ester		Trace
	Total			20.35

at which point they experienced lower body weight gains than the HFD group (31.98 ± 0.81 g *vs.* 33.39 ± 0.90 g – $p < 0.05$) until the end of the study (Week 16), resulting in 32.30% less of an increase (37.92 ± 1.71 g *vs.* 42.25 ± 1.03 g – $p < 0.05$).

Fat status was monitored with regard to the omental fat (Fig. 2B) and retroperineal fat (Fig. 2C) that were harvested from the three groups at the end of the study. In the LFD group, the omental fat and retroperineal fat weighed 0.57 ± 0.07 g and 0.16 ± 0.03 g, respectively, after 16 weeks. In the HFD group, omental and retroperineal fat rose 304% (2.30 ± 0.15 g *vs.* 0.57 ± 0.07 g – $p < 0.001$) and 286% (0.61 ± 0.13 g *vs.* 0.16 ± 0.08 g – $p < 0.001$), respectively, compared with LFD animals.

In FED-treated animals, fat in the omental and retroperineal muscles gained 17.92% (1.99 ± 0.23 g *vs.* 2.30 ± 0.15 g) and 17.78% (0.53 ± 0.06 g *vs.* 0.61 ± 0.13 g) less weight, respectively, compared with HFD mice at the end of the study. These results, however, were not statistically significant.

Changes in fasting blood glucose and fasting plasma insulin

Fasting blood glucose levels were recorded throughout the entire experiment, as reported in Fig. 3A. Fig. 3B shows the levels of fasting plasma insulin at the end of the study, Week 16.

Animals in the LFD group had normal glycemia levels during the 16-week treatment (120.15 ± 5.16 mg/dl at Week 0, and 100.20 ± 5.33 mg/dl at Week 16). In contrast, HFD animals increased fasting blood glucose levels progressively from the beginning of the study (122.15 ± 3.90 mg/dl) until Week 16 (176.85 ± 7.43 mg/dl).

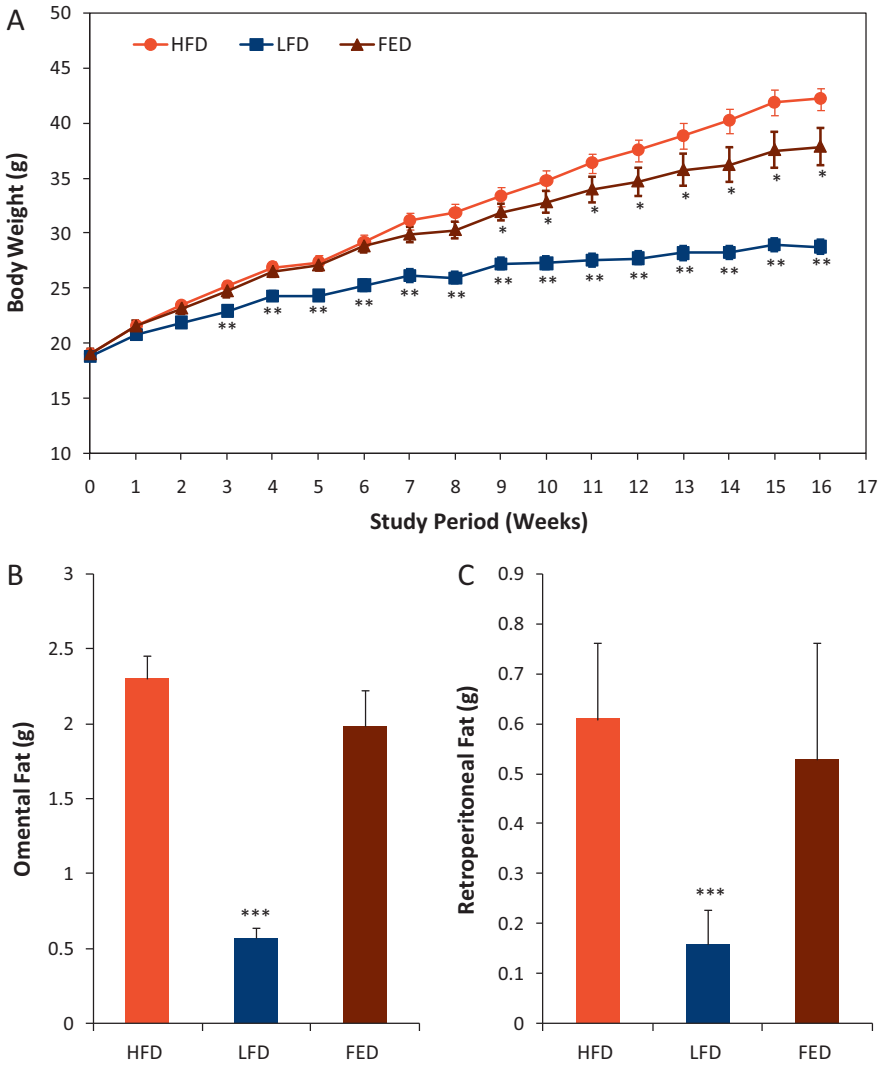


Fig. 2. (A) Changes in body weight in high-fat diet (HFD), low-fat diet (LFD), and high-fat diet + 0.5% FraxiPure™ (FED) animals over 16 weeks. (B) Changes in omental fat in HFD, LFD, and FED mice. (C) Changes in retroperitoneal fat in HFD, LFD, and FED mice. Values are mean ± SEM for LFD and HFD ($n = 20$) and FED ($n = 10$). Significance is indicated as (*) at $p < 0.05$, (**) $p < 0.001$, and (***) $p < 0.0001$ compared with the HFD control group, using the LFD negative control group as the common reference.

In the FED-treated group, fasting blood glucose rose 76.52% less (118.20 ± 9.09 md/dl – $p < 0.001$) compared with HFD mice at the end of the study. Moreover, at Week 16, FraxiPure™ reduced fasting plasma insulin levels by 53.43% in the FED-treated group compared with HFD mice (1.29 ± 0.29 ng/ml *vs.* 2.38 ± 0.29 ng/ml – $p < 0.05$).

Changes in liver weight, incidence of fatty liver, and plasma ALT levels

After 16 weeks of treatment, the average liver weight (Fig. 4A) reached 0.96 ± 0.05 g in the LFD group and increased 40.43% more in the HFD group (1.35 ± 0.09 g – $p < 0.05$). In the FED-treated group, the average liver weight increased 63.62% less compared with HFD animals (1.10 ± 0.03 g – $p < 0.05$).

Fatty livers (Fig. 4B) developed in 1 of the 20 mice in the LFD group at Week 16. This rate was 300% higher in the HFD group (4 of 20) and 66.67% lower in the FED-treated group (1 of 10) compared with HFD animals.

In addition, plasma ALT levels (Fig. 4C) in LFD animals were 22.64 ± 3.54 U/L at the end of the study, increasing 236% in the HFD group (76.20 ± 9.06 U/L – $p < 0.0001$). FED mice increased plasma ALT levels 38.01% less (55.80 ± 9.17 U/L) than HFD animals.

Discussion

Many naturally occurring phytonutrients have beneficial effects on health (Steffen 2009). In addition, several phytonutrients have received positive attention, based on their relative safety and the accumulation of evidence of their antiobesity and antihyperglycemia effects in animals and humans; these properties exist in specific flavonoids (Hwang et al. 2005), chlorogenic acid from green coffee bean (Dellalibera et al. 2006), and carnosic acid in rosemary (Takahashi et al. 2009) and have recently been proposed for secoiridoids from *F. excelsior* (Bai et al. 2010).

Our results demonstrate the substantial physiological and biochemical health benefits of a *F. excelsior* seed extract in obese mice that are fed a high-fat diet.

The differential weight gain patterns that we observed, despite similar average daily intake throughout the study, confirmed the validity of our calorie-controlled obese mouse model. Although HFD mice grew steadily throughout the study, doubling in weight, the low-calorie diet in the LFD group significantly lowered gains in weight after Week 3. Mice that were supplemented with FraxiPure™ in the FED group grew similarly to HFD animals; at Week 9, FED began to experience significant reductions in weight gain, like LFD healthy mice.

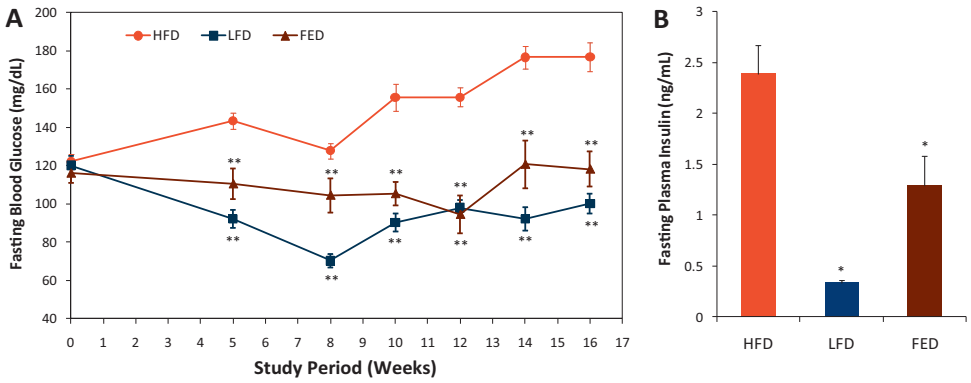


Fig. 3. (A) Changes in fasting blood glucose levels in high-fat diet (HFD), low fat-diet (LFD), and high-fat diet + 0.5% FraxiPure™ (FED) animals during the 16-week study. (B) Changes in fasting plasma insulin levels in HFD, LFD, and FED mice. Values are mean ± SEM for LFD and HFD ($n = 20$) and FED ($n = 10$). Significance is indicated as (*) at $p < 0.05$ and (**) $p < 0.001$ compared with the HFD control group, using the LFD negative control group as the common reference.

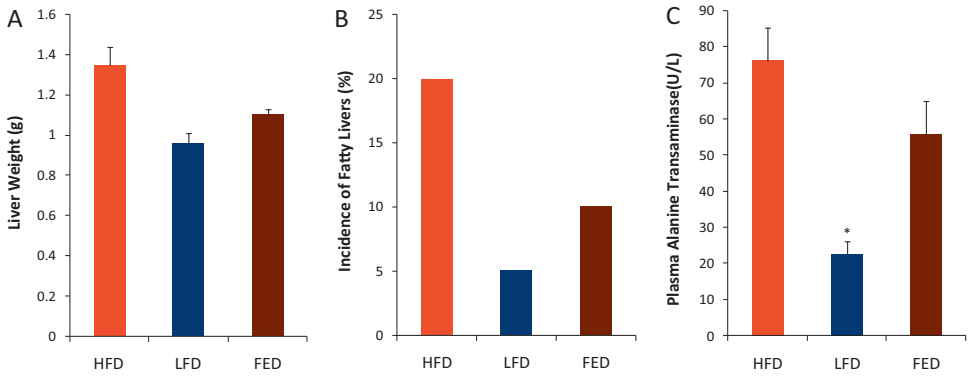


Fig. 4. (A) Changes in liver weight in high-fat diet (HFD), low-fat diet (LFD), and high-fat diet + 0.5% FraxiPure™ (FED) animals after the 16-week study. (B) Changes in fatty liver rates in HFD, LFD, and FED mice. (C) Changes in fatty plasmatic ALT levels in HFD, LFD, and FED mice. Values are mean ± SEM for LFD and HFD ($n = 20$) and FED ($n = 10$). Values are mean ± SME for HFD and LFD ($n = 20$) and FED ($n = 10$). Significance is indicated as (*) at $p < 0.05$ compared with the HFD control group.

These results complement work by Maghrani and colleagues (2004), who reported that administration of *F. excelsior* seed extract for 2 weeks (20 mg/Kg) reduced body weight in streptozotocin-induced diabetic rats but not in normal animals. Similarly, the HFD group experienced increases in adipose tissue weight compared with LFD animals over 16 weeks, while FED mice did not gain as much fat, based on omental fat, retroperitoneal fat, liver weight, and, consequently, the incidence of fatty livers, trending toward the lower levels that were observed in LFD animals.

We demonstrated recently that secoiridoid glucosides from *F. excelsior* seeds dose-dependently activate PPARα *in vitro* and inhibit preadipocyte differentiation in the 3T3-L1 cell model (Bai et al. 2010). PPARα, a transcription factor that regulates energy homeostasis (Van Raalte et al. 2004), is highly expressed in liver, heart, muscle, and kidney, where it controls fatty acid uptake and β-oxidation (Sonoda et al. 1998; Staels and Fruchart 2005) by modifying the expression of specific genes, such as acyl-CoA synthetase and fatty acid transport proteins (Schoonjans et al. 1995; Reddy and Hashimoto 2001). PPARα also increases the expression of lipoprotein lipase (LPL) and downregulates apo-C-III, an inhibitor of LPL (Staels et al. 1998).

Additionally, PPARα ligands are used widely to lower serum triglycerides and increase high-density lipoprotein cholesterol in patients with obesity, dyslipidemia, atherosclerosis, and coronary heart disease (Staels and Fruchart 2005). In our study, the decrease in fat levels in FED animals, correlating with evidence that secoiridoid glucosides from *F. excelsior* seed extracts activate PPARα and inhibit preadipocyte differentiation (Bai et al. 2010), demonstrates that FraxiPure™, due specifically to secoiridoid glucosides, has antiobesity effects *in vivo*, likely by lowering the rate of adipocyte differentiation in growing animals and enhancing fat catabolism.

In addition to these observations, with regard to weight management, if we assume that obesity is associated with increased insulin resistance (Kruszynska and Olefsky 1996), it becomes easier to understand how obesity contributes to reduced glucose uptake by muscle and liver cells (Mcgarry 1992). Consequently, local glucose storage (as glycogen) decreases, effecting triacylglycerol accumulation in adipocytes and liver cells and resulting in steatosis through the esterification of free fatty acids that are supplied primarily from the diet, coupled with increased LPL activity (Rossi et al. 2010). A notable outcome of this phenomenon is the development of type 2 diabetes (Leahy 2005), wherein insulin resistance is considered to be significantly worse than for nonobese diabetics (Seely and Olefsky 1993).

A corollary to this model can be proffered from our observations. Although significant increases in fasting blood glucose and fasting plasma insulin levels were observed after 16 weeks in obese HFD animals compared with baseline values, despite their high-fat diet: (1) fasting blood glucose in the FED group did not differ significantly from the beginning of the study, when the mice were still healthy; and (2) fasting insulin levels in FED mice decreased significantly to those of LFD animals. These observations demonstrate that FraxiPure™ has antihyperglycemia activity that is accompanied by improvements in insulin levels in FED mice, precluding the negative outcomes of obesity with regard to fasting glucose and insulin levels in HFD animals (Jeffcoat 2007). As a chief conse-

quence, the mechanisms of fat accumulation in adipocytes of FED animals are impaired and insulin resistance is improved.

These results also support previous findings of animal studies by Eddouks and Maghrani (2004) and Maghrani et al. (2004), who observed that acute intravenous administration of 10 mg/kg/h *F. excelsior* seed extract reduced blood glucose levels in normal rats for 4 h and in streptozotozin-induced diabetic rats after chronic oral consumption of 20 mg/kg/day for 14 days.

These results were galvanized when we observed a significant decrease in postprandial glycemia with humans who were administrated FraxiPure™ acutely (Visen et al. 2009). Moreover, Eddouks and Maghrani, Maghrani et al. and our group failed to note any increase in plasma insulin. Thus, the antihyperglycemia activity of *F. excelsior* seed extracts has been proposed to be caused by extrapancreatic phenomena – a hypothesis that we discussed with regard to our findings on PPARα activation (Bai et al. 2010).

To promptly evaluate liver safety, we implemented an evidence-based liver safety system, supported by changes in liver weight, fat accumulation (expressed as the rate of steatosis), and the release of ALT in plasma.

The upper limit of toxicity in this study, as monitored by steatosis rate and ALT release in the plasma, was established with data on animals in the HFD group. Compared with these results, mice that were fed a high-fat diet and administered FraxiPure™ for 16 weeks (FED) experienced significant improvements in fatty liver levels and decreases in ALT release in plasma. These findings indicate that 0.5% FraxiPure™ does not induce any side effects in mice, as monitored principally by liver physiology and biochemistry. Moreover, FraxiPure™ improved the healthy status of obese mice that were affected by a 16-week high-fat diet (the FED group).

In conclusion, this study confirms the capacity of *F. excelsior* to control body weight and supports the existing evidence of its antihyperglycemic effects and ability to improve resistance to insulin. The positive effects on weight and blood glucose might be attributed to the secoiridoids in FraxiPure™, likely through the enhancement of fat metabolism through β-oxidation, the inhibition of adipocyte differentiation during animal growth, and limited fat accumulation.

Finally, our results suggest that this botanical extract is effective, safe, and well tolerated for long periods in obese mice that are fed a high-fat diet.

We propose that secoiridoids constitute an active component in *F. excelsior* seeds, limiting weight gains and hyperglycemia through PPARα activation *in vivo*. To this end, our results encourage further study on the bioavailability of secoiridoids in FraxiPure™ and their mechanisms of regulation *in vivo*.

Author's disclosure statement

Naturex is involved in the research/development and marketing/sales of FraxiPure™ as an ingredient for the food, nutraceutical, and cosmetic industries. Therefore, Naturex has a commercial interest in this publication. The Center for Excellence in Post-Harvest Technologies (CEPHT), the conducting laboratory, was paid by Naturex to perform and report the scientific work, which formed the basis of this publication; CEPHT and Naturex declare that the data in this publication represent a true and faithful representation of the work that was performed.

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