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Comparative inhibitory effect of prenylated coumarins, ferulenol and ferprenin, contained in the ‘poisonous chemotype’ of *Ferula communis* on mammal liver microsomal VKORC1 activity.

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ABSTRACT

Two distinguishable chemotypes of *Ferula communis* have been described: the ‘nonpoisonous’ chemotype, containing as main constituents the daucane esters; and the ‘poisonous’ chemotype containing prenylated coumarins, such as ferulenol and ferprenin. Ferulenol and ferprenin are 4-oxygenated molecules such as dicoumarol and warfarin, the first developed antivitamin K molecules. Antivitamin K molecules specifically inhibit VKORC1, an enzyme essential for recycling vitamin K. This latest is involved in the activation of clotting factors II, VII, IX, X. The inhibiting effect of ferulenol on VKORC1 was shown in rat, but not for species exposed to *F. communis* while *in vivo* studies suggest differences between animal susceptibility to ferulenol. The inhibiting effect of ferprenin on VKORC1 was never demonstrated. The aim of this study was to compare the inhibiting effect of both compounds on VKORC1 of different species exposed to *F. communis*. Vitamin K epoxide activity was evaluated for each species from liver microsomes and inhibiting effect of ferulenol and ferprenin was characterized. Ferulenol and ferprenin were shown to be able to inhibit VKORC1 from all analyzed species. Nevertheless, susceptibility to ferulenol and ferprenin presented differences between species, suggesting a different susceptibility to ‘poisonous’ chemotypes of *F. communis*.

Keywords: *Ferula communis* L. (Apiaceae), Ferulenol, Ferprenin, VKORC1, inhibition constant, interspecies variability

Abbreviations : VKOR, Vitamine K epoxide reductase; VKORC1, Vitamin K epoxide reductase complex subunit 1; Vitamin K[>]O, vitamin K epoxide.

1. INTRODUCTION

Ferula communis L. (Apiaceae) is a perennial and robust weed, native to the Mediterranean basin. Its presence was reported in Syria, Turkey, North Africa, Italy, Sardinia, Portugal, Greece, Croatia, Albania and Palestine (Cauwet Marc, 1981; Infante, 1965). Consumption of *F. communis* L. has been reported to be associated to an hemorrhagic syndrome, also called ferulosis, often fatal in the absence of treatment (Benzaldi, 1988; Cauwet Marc 1990). This intoxication affected almost all mammals and the National Center of Veterinary Toxicology of Lyon (France) diagnosed 28 clinical cases of *Ferula communis* intoxication in cattle, sheep, pig and horse from 1990 to 2013 (Gault et al, 2013). Even man may be affected after an uncontrolled therapeutic use of *Ferula* extract (Cornevin et al, 1887; Lannehoa et al, 1998).

Two distinguishable chemotypes of *F. communis* L. were reported: the ‘non-poisonous’ chemotype and the ‘poisonous’ chemotype associated to the hemorrhagic syndrome (Benkhalti and Lamnaouer, 1994; Sacchetti et al, 2003). Different chemical investigations have reported the presence of daucane esters or drimane ethers, as main constituents in the ‘non-poisonous’ chemotype, according to the geographic area and the presence of prenylated coumarins as main constituents in the ‘poisonous’ chemotype (Appendino et al, 1987; Appendino et al, 2001; Fraigui et al, 2001; Miski and Mabry, 1985; Valle et al, 1987; Rubiolo et al, 2006). Among the prenylated coumarins of the toxic variety, both ferulenol, a 4-hydroxycoumarin derivative, and ferprenin, a pyrane (3,2-c) coumarin derivative (Appendino et al, 1988; Carboni et al, 1964) (Figure 1) could affect blood clotting, as described for other 4-oxygenated coumarin derivatives such as dicoumarol or warfarin, the first developed antivitamin K molecules used in human medicine and as rodenticide for pest control.

4-hydroxycoumarin derivatives, also designed as antivitamin K molecules were reported to specifically inhibit VKORC1, an enzyme encoded by the recent discovered *Vkorc1* gene (Li

1 et al, 2004; Rost et al, 2004). This enzyme catalyzes the vitamin K 2,3-epoxide reductase
2 activity. This enzymatic activity is essential for recycling vitamin K. The function of
3
4 VKORC1 is to regenerate vitamin K and vitamin K hydroquinone (K and KH₂) from vitamin
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6 K 2,3-epoxide (K>O), a byproduct of the vitamin K-dependent gamma carboxylation reaction
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8 (Oldenburg et al, 2008). Inhibition of VKORC1 by 4-hydroxycoumarin derivatives limits the
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10 amount of KH₂ available for the carboxylation reaction and results in partially carboxylated
11
12 vitamin K-dependent blood clotting factors II, VII, IX, X. The specific inhibition of VKORC1
13
14 results in a stop of the clotting factors activation leading to a delayed death by hemorrhage.
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16 The inhibiting effect of ferulenol on VKORC1 was shown in rat (Gebauer et al.), but not on
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18 VKORC1 of species exposed to *F. communis* L..

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24 Previous studies in sheep treated with ferulenol indicated a sharp decreased in activity for
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26 several coagulation factors (Tligui et al, 1994). This observation seems to be coherent with
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28 the VKORC1-mediated antivitamin K properties of 4-oxygenated coumarins. Nevertheless,
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30 another mechanism leading to decrease in coagulation factors was proposed. This decrease
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32 was proposed to be due to an impairment of coagulation factors biosynthesis induced by a
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34 cytotoxic effect of ferulenol (Monti et al, 2007). Moreover, the toxicity of *F. communis* L.
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36 was reported to be not correlated with its contents in ferulenol (Appendino et al, 1988) and
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38 another prenylated coumarin, the pyranoferprenin, isolated from *F. communis* L., showed in
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40 vivo haemorrhagic activity (Appendino et al, 1988). Nevertheless, the inhibiting effect of
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42 ferprenin on VKORC1 was never demonstrated. This study aims to compare the inhibiting
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44 effect of ferulenol and ferprenin on VKORC1 from different species in order to demonstrate
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46 the mode of action of phytochemicals presumed to be responsible for the toxicity of *F.*
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communis, plant associated to intoxication that has considerable importance in plant/animal
interactions in the Mediterranean countries.

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9 **2. RESULTS**

10 **2.1.Determination of vitamin K epoxide reductase activity in liver of animal species**
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12 **susceptible to be exposed to *F. communis* L.**
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16 In order to evaluate the vitamin K recycling ability of animal species exposed to *F. communis*
17 L., liver microsomes were prepared from cow, calf, goat, lamb, pig, boar and horse. VKOR
18 activity was thus measured at saturating concentration of vitamin K epoxide substrate (i.e.,
19 200 μ M) from the various microsomal fractions. Figure 2 presents the results obtained. All the
20 liver microsomal fractions tested in this study were able to reduce vitamin K epoxide in
21 vitamin K. VKOR activities were found statistically different between species (VKOR
22 activities determined from horse and boar were excluded of the one way analysis of variance).
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24 VKOR activity measured in cow liver microsomes was 1.5 to 2-fold higher than that
25 measured in goat, lamb and pig liver.
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41 **2.2.Determination of the inhibiting effect of ferulenol and ferprenin on vitamin K**
42 **epoxide reductase activity**
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45 In order to analyze the efficiency of ferulenol and ferprenin as inhibitor of the VKOR activity,
46 susceptibilities to ferulenol and ferprenin were first determined in the presence of calf liver
47 microsomes. The plots of the velocity of the VKOR activity catalysed by calf liver
48 microsomes, versus the substrate concentration in the presence of different concentrations of
49 ferulenol or ferprenin are presented in Figure 3A and 3B, respectively. The inhibiting effects
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1 of ferulenol and ferprenin on VKOR activity catalysed by calf liver microsomes were
2 compared with that obtained in the same conditions with warfarin (Figure 3C).
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4 Ferulenol was able to inhibit VKOR activity. The addition of ferulenol did not modify the
5 apparent K_m ($40.3 \pm 5 \mu\text{M}$), while it decreased the apparent V_{max} (Figure 3A). Ferulenol is
6 thus able to inhibit VKOR activity catalysed by calf liver microsomes in a non-competitive
7 manner, as observed with warfarin (Figure 3C). Data were fitted to the Michaelis–Menten
8 model, which takes into account the presence of either competitive, non-competitive, or
9 uncompetitive inhibitor by non-linear regression. A fit was possible when the model that takes
10 into account a non-competitive inhibitor was used only. Finally, K_i towards ferulenol for calf
11 liver microsomes was $0.076 \pm 0.007 \mu\text{M}$. K_i towards warfarin obtained in the same conditions
12 was $0.51 \pm 0.04 \mu\text{M}$.
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26 Ferprenin was also able to inhibit the VKOR activity catalyzed by calf liver microsomes, but
27 with concentration much higher than those used for inhibition of VKOR activity by ferulenol
28 (Figure 3B). Ferprenin inhibited the VKOR activity in a non-competitive manner, as
29 described for ferulenol. K_i towards ferprenin for calf liver microsomes was $8.2 \pm 0.05 \mu\text{M}$.
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2.3.Determination of the species variability of the VKORC1-inhibiting effect of ferulenol

In order to analyse the variation of the efficiency of ferulenol as inhibitor of VKOR activity in
the animals exposed to *F. communis L.*, K_i values for ferulenol were determined in liver
microsomal fractions from cow, goat, lamb, pig, horse and boar and compared to K_i obtained
for rat liver microsomes. Ferulenol inhibited the VKOR activity in a non-competitive manner
for all the microsomal fractions we analysed (data not shown). The K_i values for ferulenol are

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in Table 1. They were found statistically different between species (K_i determined from-horse and boar were excluded of the one way analysis of variance). K_i for ferulenol determined in cow liver microsomes was 3- to 3.8-fold higher than that measured in calf, goat, lamb, pig and rat liver.

2.4.Determination of the species variability of the VKORC1-inhibiting effect of ferprenin

In order to analyse the variation of the efficiency of ferprenin as inhibitor of VKOR activity in the animals exposed to *F. communis* L., K_i values towards ferprenin were determined in liver microsomal fractions from cow, goat, lamb, pig, horse and boar and compared to K_i obtained for rat liver microsomes. Ferprenin inhibited the VKOR activity in a non-competitive manner for all the microsomal fractions we analysed (data not shown). The K_i values for ferprenin are in Table 1. They were found statistically different between species (K_i determined from horse and boar were excluded of the one way analysis of variance). K_i for ferprenin determined in cow liver microsomes was 3.2-, 6.0-, 2.1-, 1.6- and 9.0-fold higher than that measured in calf goat, lamb, pig and rat liver.

2.5.Determination of the species variability of the VKORC1-inhibiting effect of warfarin

In order to compare the efficiency of ferulenol and ferprenin as inhibitor of VKOR activity to that of warfarin, K_i for warfarin were determined in liver microsomal fractions from cow, goat, lamb, pig, horse and boar. The K_i values are in Table 1. They were found statistically different between species (K_i determined in horse and boar were excluded of the one way analysis of variance).

3. DISCUSSION

Ferulenol and ferprenin are 4-oxygenated coumarin derivatives synthesized by *F. communis* L.. Coumarin derivatives are known to be potential inhibitor of vitamin K epoxide reductase activity responsible for the recycling of vitamin K, a cofactor essential for the coagulation process. The presence of ferulenol and/or ferprenin was therefore associated with the toxicity of the ‘poisonous’ chemotype of *F. communis* L. (Appendino et al, 1988; Fraigui et al, 2001). Indeed, the symptomatology associated with *F. communis* L. poisoning is coherent with an anti-VKORC1 activity of ferulenol and/or ferprenin. Nevertheless, few studies allowing to prove the role of ferulenol and/or ferprenin in ‘ferulosis’ are available. The involvement of ferulenol in the hemorrhagic syndrome was shown by in vivo studies in rats (Aragno et al, 1988, Fraigui et al, 2001, Fraigui et al, 2002, Lamnaouer et al, 1991; Tagliapietra et al, 1989), mice (Fraigui et al, 2001, Fraigui et al, 2002), sheep (Lamnaouer et al, 1990; Otieno et al, 1979; Shlosberg et al, 1985) and lamb (Egber et al, 1998). Nevertheless, the mechanism was not described, except that consumption of ferulenol was associated to decrease in vitamin K-dependent clotting factors. The unique study demonstrating the in vitro inhibiting effect of ferulenol on VKORC1 was performed in rat (Gebauer, 2007), while inter-species differences might exist, since the replacement of one single amino-acid of VKORC1 can lead to enzyme resistant to coumarin derivatives (Grandemange et al, 2010, Hodroge, 2011). The inhibiting effect of ferulenol on VKORC1 of farm animals is still unknown.

In order to determine the underlying mechanism of ‘ferulosis’, VKOR activity was first analyzed in animal species exposed to *F. communis* L.. VKOR activity has been well characterized in rodents because of the intensive use of coumarin derivatives as rodenticides. In rodents, this activity is exclusively catalyzed by VKORC1 in liver, while this activity might be catalyzed by two enzymes, VKORC1 and VKORC1L1, in other tissues. In farm

1 animals, VKOR activity was until now never studied. Liver microsomes from cow, calf, goat,
2 lamb, pig, boar and horse were used to examine VKOR activity by a DTT-driven VKOR
3 assay, as previously described (Hodroge et al, 2011). This experimental method is a direct
4 approach based on the measurement of the VKORC1 reaction product, the vitamin K. By
5 using this method, VKOR activity was detected in liver of all species analyzed in this study.
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7 Nevertheless, variations of VKOR activity between species were observed. VKOR activity
8 measured in cattle liver was 1.5- to 2-fold higher than in other species, suggesting inter-
9 species variation in the vitamin K recycling ability. VKOR activity measured in the liver of
10 the different species was efficiently inhibited by warfarin with K_i about 1 to 2 μM , as
11 observed for rats. The inhibition of VKOR activity by warfarin observed in this study is thus
12 totally coherent with an exclusive involvement of VKORC1 in hepatic VKOR activity of the
13 different species exposed to *F. communis* L., as described for rats (Hammed et al, 2013).
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31 Since VKOR activity was detected in liver of all animals used in this study, effect of ferulenol
32 and ferprenin on this VKOR activity was analyzed. Ferulenol and ferprenin were found in this
33 study to be able to inhibit VKOR activity in a non-competitive manner, as described for
34 warfarin. The mechanism of inhibition proposed by Silverman (1980) implies the acylation by
35 the 4-oxygenated coumarins of one Cys of the catalytic site of VKORC1 or possibly of a
36 nearby nucleophile amino acid. The proposed mechanism agrees with the irreversible
37 characteristic of the inhibition of the VKOR activity by ferulenol and ferprenin observed in
38 this study.
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50 Ferulenol was already reported to inhibit rat VKORC1, with K_i of 0.098 μM (Gebauer, 2007).
51 In this study, inhibition of rat VKORC1 by ferulenol was highly similar to that previously
52 reported, with a K_i of 0.04 μM . K_i obtained for ferulenol is 10-fold lesser than that obtained
53 for warfarin (Figure 4). Ferulenol is thus a more potent inhibitor of VKOR activity than
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1 warfarin, which is traditionally used in human medicine for patients requiring anticoagulation
2 therapy. K_i obtained for ferulenol is similar to those obtained for the most powerful molecules
3 used as rodenticides such as difethialone or brodifacoum (Hodroge et al, 2011). Ferulenol is
4 also able to inhibit VKORC1 of other species, with K_i similar to that obtained for rat, despite
5 the variations of VKORC1 amino acid sequences between species (i.e., identities between
6 VKORC1 amino acid sequences are comprised between 68 and 84%) (Figure 5). Surprisingly,
7 VKORC1 of cow seems to be more resistant to ferulenol than other VKORC1, while it
8 presents the highest amino acid sequence identity with rat VKORC1. Nevertheless,
9 susceptibility to ferulenol can be qualified to be similar between rat and farm animals. This
10 result is coherent with published studies showing that repeated administration of ferulenol
11 was able to lead to increase in prothrombin time and in reduction of pool of vitamin K-
12 dependent clotting factors (Aragno et al, 1988, Fraigui et al, 2001; Shlosberg et al, 1985;
13 Tagliapietra et al, 1989).

34 When ferprenin was orally given to rats, Aragno et al (1988) indicates that this compound
35 clearly increases prothrombin time at very high dosage (a minimum of $30 \text{ mg}\cdot\text{kg}^{-1}$ for 3 days
36 or $10 \text{ mg}\cdot\text{kg}^{-1}$ for 5 days). Using DTT-driven VKOR assay, ferprenin was also found to be
37 able to inhibit VKOR activity in liver of all species analyzed in this study. Contrary to
38 ferulenol, this ability to inhibit VKOR activity is very surprising, because a free 4-
39 hydroxycoumarin moiety was described to be necessary for anti-VKORC1 activity.
40 Nevertheless, K_i obtained for ferprenin were 2 to 10-fold higher than those obtained for
41 warfarin (Figure 4) and ferulenol is 100- to 500-fold more potent to inhibit VKOR activity
42 than ferprenin, according to the species (Figure 4). Ferprenin is thus a very bad inhibitor of
43 VKOR activity for all species analyzed in this study. The direct effect of this molecule
44 remains surprising because the 4-OH moiety of the coumarinic ring appears to be essential for

1 the interaction with VKORC1 enzyme as shown by Gebauer (2007). The hydrolysis of the
2 pyrane cycle of ferprenin is considered by Aragno et al (1988) as impossible nevertheless it
3 should be interesting to evaluate this possibility either in vivo or even during the microsomal
4 DTT-driven VKOR assay. Contrary to ferulenol, ferprenin seems to be more subject to inter-
5 species variation. Indeed, in rats, K_i obtained for ferprenin is about 1 μM , while in cow K_i is
6 about 15 μM . Therefore, ferprenin could be much more potent to inhibit VKORC1 in rat than
7 in cow. Moreover, demonstration of in vivo effect of ferprenin is only available in rat (Aragno
8 et al, 1988).

21 4. CONCLUSIONS

22 Ferulenol, and in a lesser extent, ferprenin are thus certainly both responsible for the
23 hemorrhagic syndrome. Nevertheless, *F. communis* also contain COX-inhibiting
24 polyacetylenes that could synergistically contribute to the poisonous property of the plant
25 (Appendino et al, 1993). Toxicity of ferulenol and ferprenin is also driven by
26 pharmacokinetics and inter-species variations in pharmacokinetics of these compounds. Few
27 studies about pharmacokinetics of these compounds are available. Published results argue for
28 a high metabolic clearance of ferulenol. Indeed, while K_i obtained for ferulenol is lower than
29 that obtained for warfarin, the dosage of ferulenol necessary to lead to an increase in
30 prothrombin time in rat appears to be about 10-fold higher than that used for warfarin (Aragno
31 et al, 1988). The hemorrhagic effect is strictly dependent on the continuous presence of high
32 concentration of VKORC1 inhibitor in the liver. Indeed, few hours without this inhibition will
33 be enough to fully restore the pool of clotting factors. Considering a high metabolic clearance,
34 the toxicity of such compounds will directly depend on the pattern of administration. Frequent
35 repeated dosages are strictly necessary to maintain the inhibiting concentration in the liver.
36 Ruminal administration can be considered as a continuous delivery of ferulenol even if the

1 consumption of *F. communis* L. is not continuous as observed in sheep after oral
2 administration of repeated administration of ferulenol. (Tligui et al, 1994). The detection of
3 ferulenol in ‘non-poisonous’ chemotypes of *F. communis* L. (Rubiolo et al, 2006) questioned
4 the only responsibility of ferulenol and ferprenin in the hemorrhagic syndrome. Nevertheless,
5 ferulenol and ferprenin are found to be present in far smaller amounts in ‘non-poisonous’
6 chemotypes of *F. communis* L. than in ‘poisonous’ chemotypes. The consumption of these
7 chemotypes does not allow to reach sufficient hepatic concentration leading to complete
8 inhibition of VKOR activity in liver and thus in a stop of the vitamin K-dependent clotting
9 factors activation.
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24 **5. MATERIALS AND METHODS**

25 **5.1. Animals**

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32 Livers from lamb, cow, horse, goat, pig and calf were collected from the slaughterhouse at
33 Saint Romain de Popey (France) under special authorization of the veterinary services and
34 after veterinary health examination. Male OFA-Sprague Dawley rats (200g) were obtained
35 from a commercial breeder (Charles River, L’Arbresles, France) and acclimated for a minimal
36 period of 5 days. Rat food and water were available ad libitum. The rats were killed by
37 decapitation. The boar was hunted and the liver was removed according to the regulations.
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39 All samples were immediately frozen at - 80° C. Three different animals of each species were
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54 **5.2. Chemical syntheses**

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1 trans,trans-farnesol with PDC afforded farnesal in 87% yield (Hu et al, 2004). Ferprenin was
2 obtained by reaction of 4-hydroxycoumarin with 2 equivalents of trans,trans-farnesal in water
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4 at 80°C, in a Knoevenagel / electrocyclization tandem mechanism (Jung et al, 2010) (SI 1).
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9 **5.3. Microsomes preparation**

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11 Liver microsomes were prepared by differential centrifugations as previously described
12 (Moroni et al, 1995). Protein concentrations were determined by the method of Bradford
13 (1976) using serum albumin as a standard.
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21 **5.4. Vitamin K epoxide reductase activity**

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23 Microsomal vitamin K epoxide reductase (VKOR) activity was assayed according to a
24 modified protocol previously described by Thijssen (1987), Thijssen and Baars (1989) and
25 Misenheimer and Suttie (1990). Briefly, standard reactions were performed in 200 mM Hepes
26 buffer (pH 7.4) containing 150 mM KCl, 1 mM dithiothreitol, 1 mg of total proteins. The
27 reaction was started by the addition of vitaminK₂O solution in 1% Triton X-100. After
28 incubation at 37°C for 30 min, the reaction was stopped by adding 4 mL of iced 1:1
29 isopropanol/hexane solution. After centrifugation at 5000g for 10 min, the hexane layer was
30 removed and dried under nitrogen. The dry residue was immediately dissolved in 0.2 mL of
31 isopropanol and reaction product was analyzed by UV-HPLC.
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48 **5.5. Vitamin K1 measurement**

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50 Vitamin K1 was measured by high performance liquid chromatography (HPLC). The HPLC
51 chain consisted of an autosampler (Merck AS-2000A), an intelligent pump (Merck L-6200A),
52 a UV-VIS detector (Merck L-4250) and an enregistor (Merck SS420X). Separation was
53 achieved on a C8, 5 µm, 4,6 x 150 mm analytical column (Sunfire, Irl). The flow rate of the
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1 mobile phase (methanol 96%, water 4%, formic acid 1%) was 0.8 mL/min. The column
2 temperature was 40°C. Detection was performed at 248 nm.
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6 7 **5.6.Kinetics**

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9 Kinetic parameters (K_m and V_{max}) were obtained from at least two separate experiments after
10 the addition of increasing amounts of vitamin K₂O (0.003 to 0.2 mM) to the standard
11 reaction. The estimation of the kinetic parameters was achieved by the incubation of at least 9
12 different concentrations of vitamin K₂O. Incubations were performed in duplicate. Data were
13 fitted by nonlinear regression to the Michaelis-Menten model using the curve fitter program
14 of Sigmaplot 9.0TM software.
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17 In order to evaluate the inhibiting effect of various molecules (warfarin, ferulenol and
18 ferprenin) (figure 1) on VKOR activity, inhibition parameters (K_i) were determined after
19 addition of various concentrations of molecules to the standard reaction. Inhibition parameters
20 were first assessed with 4 different concentrations (0.01, 1, 10 and 30 μ M) and further more
21 precisely determined using concentrations from about 0.05 to $20 \times K_i$. Data were fitted by non-
22 linear regression to the non-competitive inhibition model using Sigmaplot 9.0TM software.
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42 This work was supported by grants ISI n°I1301001W “NEORAMUS” from Bpi France.
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50 **LEGENDS OF FIGURES**

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53 **Figure 1:** Chemical structures of warfarin (A), Ferulenol (B) and Ferprenin (C)
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56 **Figure 2 :** Vitamin K epoxide reductase activity in animals exposed to *F. communis*. L.
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59 Enzyme activity was evaluated in the presence of 200 μ M of KOX and 1 mg of liver
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microsomal proteins. Each data point represents the mean \pm SD of three individual determinations.

Figure 3 : Plots of vitamin K epoxide reductase activity versus vitamin K > O (6.25–200 μ M), in the presence of various concentrations of ferulenol (A) or ferprenin (B) or warfarin (C) incubated with calf liver microsomes.

Figure 4 : Comparison of the inhibition effect of warfarin, ferulenol and ferprenin using various liver microsomes. A/ Inhibition effect of ferulenol or ferprenin compared to warfarin, B/ Inhibition effect of Ferulenol compared to ferprenin.

Figure 5 : Multiple sequence alignment of VKORC1 enzymes of animals exposed to *F. communis* L. The active site is indicated by the box.

SI 1 : Ferprenin synthesis

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Table 1 : K_i values of ferulenol, ferprenin and warfarin determined in various liver microsomes.

Species	Ki for Ferulenol (μM)	Ki for Ferprenin (μM)	Ki for Warfarin (μM)
Cow	0.13 ± 0.06	16.6 ± 1.2	2.3 ± 0.4
Calf	0.04 ± 0.01	5.2 ± 1.4	2.5 ± 0.1
Goat	0.04 ± 0.02	2.8 ± 1.4	1.6 ± 1.3
Lamb	0.03 ± 0.01	8.0 ± 4.0	0.8 ± 0.2
Pig	0.04 ± 0.01	10.3 ± 1.9	1.2 ± 0.2
Boar	0.08 ± 0.02	3.7 ± 0.6	0.8 ± 0.1
Horse	0.04 ± 0.01	16.3 ± 2.1	1.3 ± 0.3
Rat	0.03 ± 0.01	1.9 ± 0.4	0.5 ± 0.1

Figure

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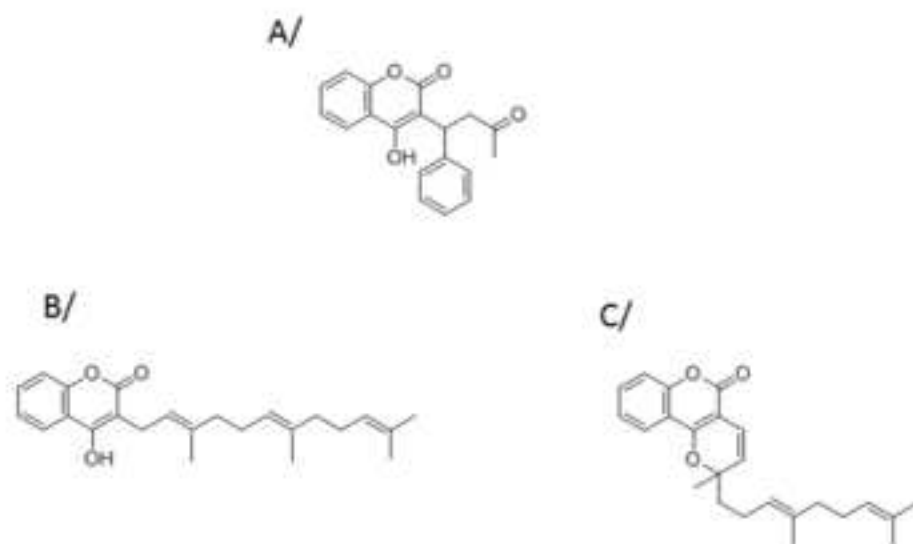


Figure 1

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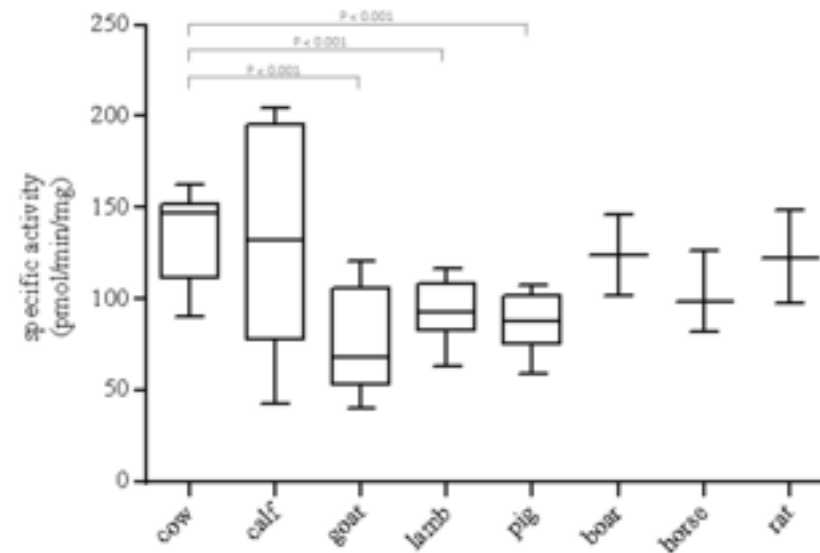


Figure 2

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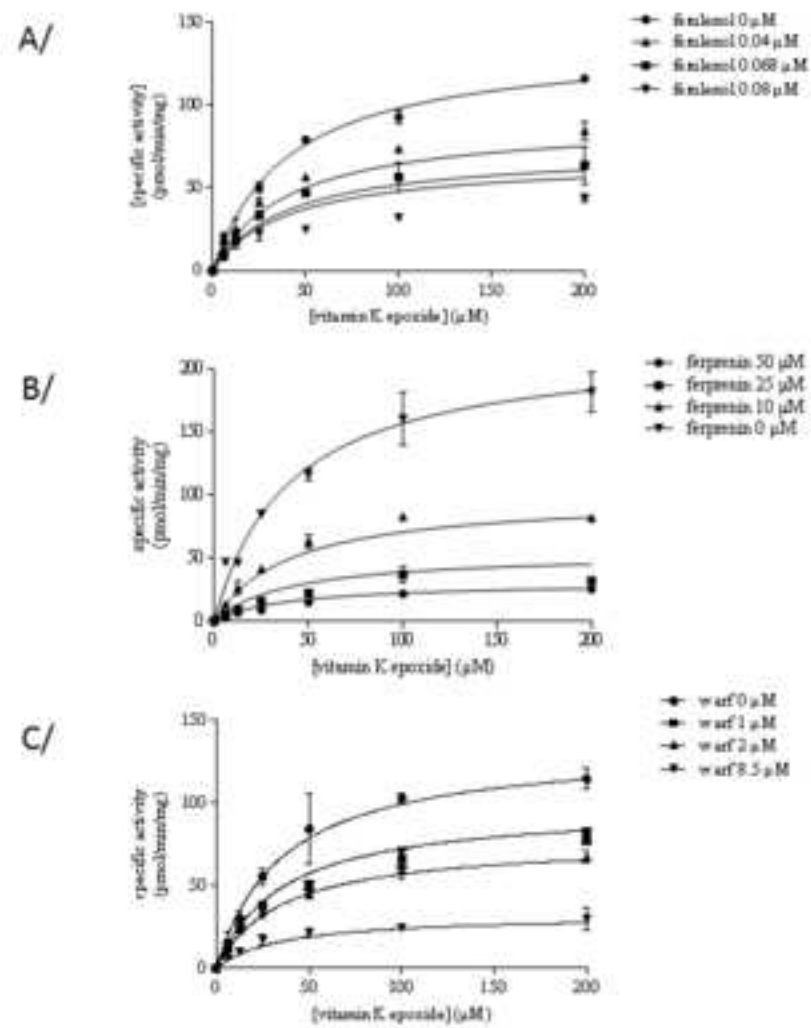


Figure 3

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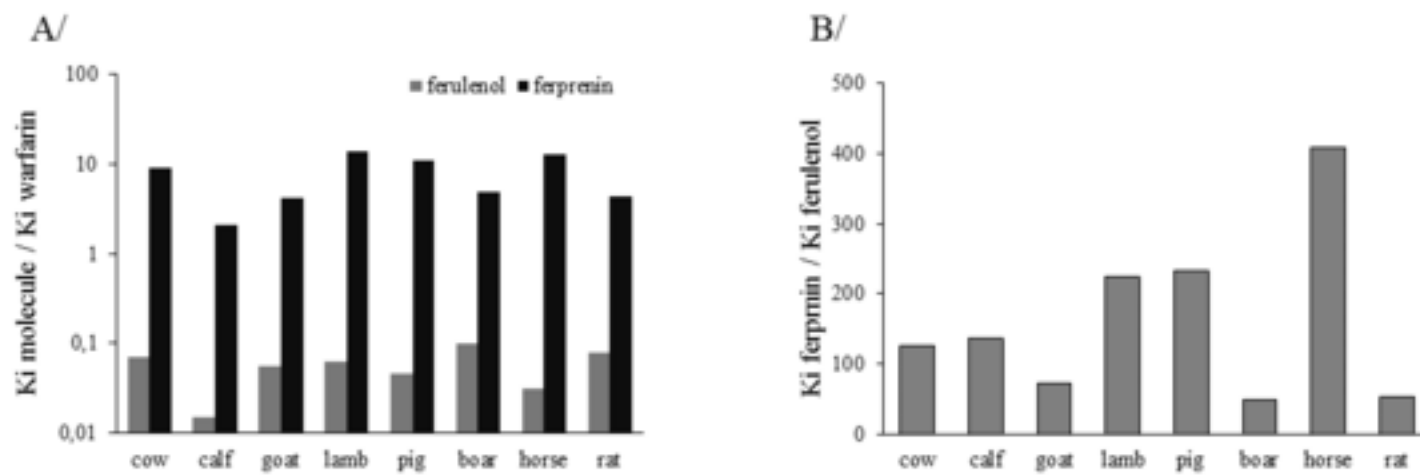


Figure 4

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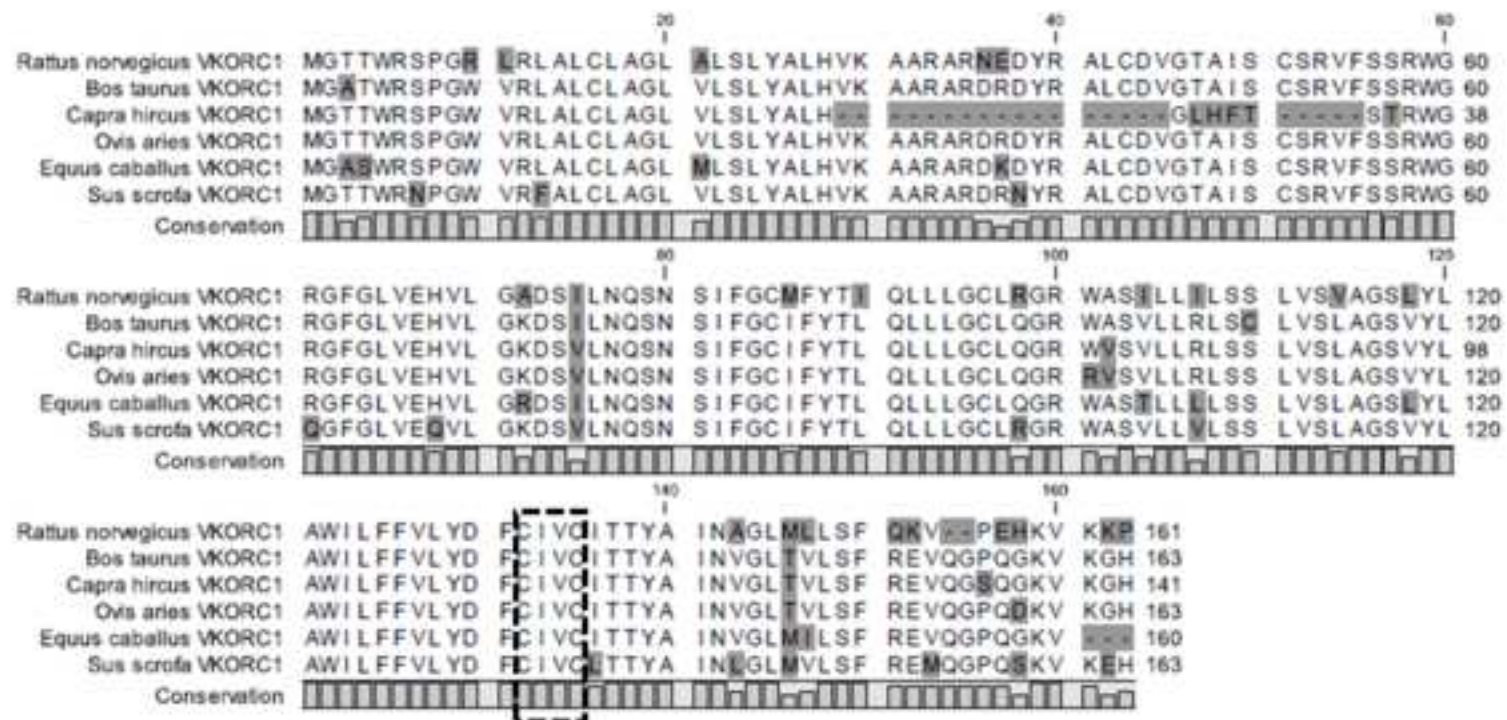


Figure 5

Supplementary Information

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