Food and Chemical Toxicology 130 (2019) 32–43

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

# Effects of short-term saffron (*Crocus sativus* L.) intake on the *in vivo* activities of xenobiotic metabolizing enzymes in healthy volunteers

Elias Begas[a], Maria Bounitsi[a], Thomas Kilindris[b], Evangelos Kouvaras[a], Konstantinos Makaritsis[c], Demetrios Kouretas[d], Eftihia K. Asprodini[a,*]

[a] Laboratory of Pharmacology, University of Thessaly, 41500, Biopolis, Larissa, Greece
[b] Laboratory of Medical Informatics, University of Thessaly, 41500, Biopolis, Larissa, Greece
[c] Department of Internal Medicine, Faculty of Medicine, University of Thessaly, 41500, Biopolis, Larissa, Greece
[d] Laboratory of Animal Physiology – Toxicology, Department of Biochemistry-Biotechnology, University of Thessaly, 41500, Biopolis, Larissa, Greece

ARTICLE INFO

*Keywords:*
Saffron
CYP1A2
CYP2A6
Xanthine oxidase
NAT2

ABSTRACT

*Crocus sativus* L., a perennial plant grown mainly around the Mediterranean and Iran, has many medicinal properties including anti-inflammatory, anti-depressive and cancer preventing properties. Aqueous herbal extracts may affect the activity of Phase I and II enzymes involved in xenobiotic metabolism. The present study was designed to determine whether *C. sativus* infusion alters the activity of CYP1A2, CYP2A6, XO and NAT2 enzymes in humans.

Thirty-four healthy volunteers consumed infusion prepared from *C. sativus* stigmata for six days. Enzyme phenotyping was assessed in saliva and urine using caffeine metabolite ratios as follows: CYP1A2: 17X/137X (saliva) and CYP1A2: (AFMU + 1U + 1X)/17U, CYP2A6: 17U/(17U + 17X), XO: 1U/(1U + 1X) and NAT2: AFMU/(AFMU + 1U + 1X) (urine).

Following *C. sativus* intake, CYP1A2 index was reduced by ~13.7% in saliva (before: 0.51 ± 0.22, after: 0.44 ± 0.14; $p = 0.002$) and ~6.0% in urine (before: 3.81 ± 1.20, after: 3.58 ± 0.92; $p = 0.054$). CYP1A2 index was significantly reduced only in males (saliva, before: 0.65 ± 0.22, after: 0.51 ± 0.16; $p = 0.0001$; urine, before: 4.53 ± 1.19, after: 4.03 ± 0.87; $p = 0.017$) suggesting sexual dimorphism in CYP1A2 inhibition. There was no effect of *C. sativus* intake on CYP2A6, XO or NAT2 indices.

Short-term consumption of *C. sativus* infusion is unlikely to result in significant herb-drug interactions involving the enzymes studied, with the exception of potential herb-CYP1A2 substrate interaction in males.

## 1. Introduction

*Crocus sativus* L. *(C. sativus)* is a perennial plant of the Iridaceae family grown in regions around the Mediterranean, Iran, India and China. Saffron is a spice derived from the dried red style branches, stigmas, of *C. sativus* flower. It is used as food additive for flavoring and coloring and is one of the most expensive spices reflected by the labor intensiveness and costs associated with its production. Although the world's total annual production of saffron originates from Iran, the largest European saffron production is Greece (Schmidt et al., 2007) where its cultivation dates back as early as 16th century B.C. as documented by Minoan frescos brought to light in Santorini and Crete. Saffron consists of a complex mixture of volatile and non-volatile compounds. The main bioactive compounds of saffron, derived from the oxidative cleavage of the carotenoid zeaxanthine, are crocin and its aglycone crocetin, which are responsible for the colouring properties of saffron, and picrocrocin and its aglycone safranal which give saffron its bitter flavour and characteristic aroma, respectively (Fig. 1). Other constituents such as mineral agents, anthocyanins, flavonoids and kaempferol have also been reported (Tarantilis et al., 1995; Papandreou et al., 2006; Sánchez et al., 2008; Sobolev et al., 2014; Karkoula et al., 2018). Apart from its value as a food additive and dye material, saffron has been also used as an effective herbal medicine. Its constituents exhibit a spectrum of beneficial pharmacological effects (Alavizadeh and Hosseinzadeh, 2014) such as anti-nociceptive, anti-inflammatory and antioxidative effects (Assimopoulou et al., 2005; Papandreou et al., 2006, 2011), neuroprotective (Linardaki et al., 2013) and memory improving effects (Pitsikas and Sakellaridis, 2006; Pitsikas et al., 2007;

* Corresponding author. Laboratory of Pharmacology, Faculty of Medicine, University of Thessaly, 41500, Biopolis, Larissa, Greece.
*E-mail addresses:* begas@med.uth.gr (E. Begas), mbounitsi@yahoo.gr (M. Bounitsi), tomkil@uth.gr (T. Kilindris), ekouvar@med.uth.gr (E. Kouvaras), makarits@med.uth.gr (K. Makaritsis), dkouret@uth.gr (D. Kouretas), easpro@med.uth.gr (E.K. Asprodini).

https://doi.org/10.1016/j.fct.2019.05.013
Received 10 April 2019; Received in revised form 8 May 2019; Accepted 9 May 2019
Available online 10 May 2019
0278-6915/ 

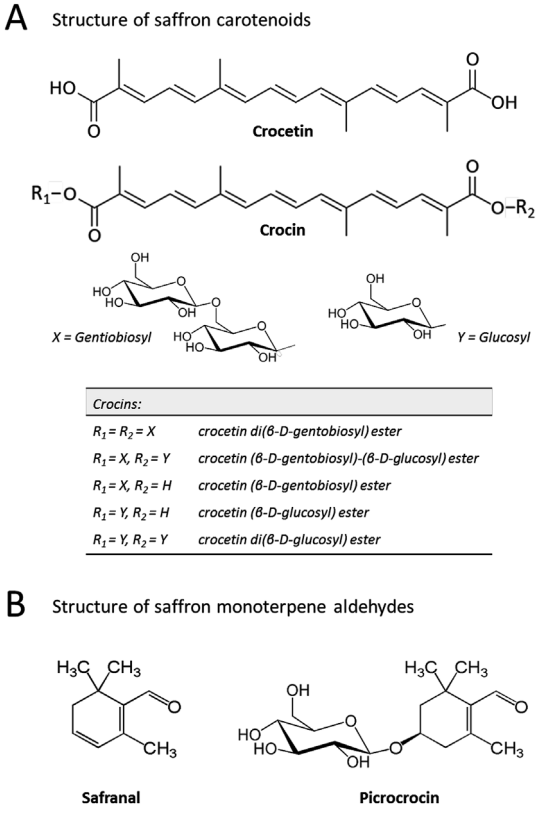


**Fig. 1. Chemical structures of main bioactive compounds from the dried stigmas of *C. sativus* L. A.** Chemical structures of saffron polyene dicarboxylic acid crocetin and its glycosylated esters crocins, responsible for the strong yellow-red color of saffron. The central unit of crocetin consists of seven conjugated double bonds and four chain methyl groups. One or both of the end groups are esterified with gentiobiose or glucose to crocins; the presence of glycosyl in crocins confers upon their high water solubility. **B.** Chemical structure of the monoterpene aldehyde safranal, responsible for the aroma of saffron, and its glycoside form picrocrocin, a monoterpene glycoside precursor of safranal, largely responsible for the bitter taste of saffron. Safranal is formed by the hydrolysis of picrocrocin during drying and storage of saffron.

Pitsikas, 2015) and has been evaluated as a potential agent for depression (Wang et al., 2010; Ghasemi et al., 2015) and cancer prevention (Tavakkol-Afshari et al., 2008; Mousavi et al., 2009; Zhang et al., 2013; Hoshyar and Mollaei, 2017; Khorasanchi et al., 2018).

The Cytochrome P450 (CYP) enzyme superfamily, present in the smooth endoplasmic reticulum of the liver and other extrahepatic tissues, is involved in the metabolism of endogenous compounds and xenobiotics such as drugs or environmental chemicals. Within the CYP superfamily, only isozymes belonging to the subfamily of CYP1, 2, and 3 are responsible for the biotransformation of most foreign substances including approximately 70% of all drugs in clinical practice (Pelkonen et al., 2008). CYP1A2, is the only hepatic member of the CYP1 family and it accounts for the 13% of the total hepatic content of cytochrome isoenzymes (Shimada et al., 1994). It is involved in the metabolism of drugs such as clozapine, olanzapine and theophylline (Pelkonen et al., 2008) as well as the metabolic activation of several procarcinogens, including aromatic and heterocyclic amines, nitroaromatic compounds, and mycotoxins (Pelkonen et al., 2008). The wide inter-subject variability observed in CYP1A2 activity has been attributed to factors such as gender, race and exposure to inducers or inhibitors, while the role of the genetic polymorphisms identified to date is questionable (Jiang et al., 2006; Pelkonen et al., 2008). Inter-individual variations in CYP1A2 activity may influence the therapeutic efficacy of some drugs and even the susceptibility to cancer risk (Le Marchand et al., 1997; Zhou et al., 2010).

CYP2A6 has a predominant role in the overall metabolism of nicotine and its metabolite cotinine as well as compounds that are of toxicological significance such as nitrosamines and aflatoxin B1 (Pelkonen et al., 2008; Di et al., 2009). Due to its low levels in human liver (~4%) (Shimada et al., 1994) its contribution to overall drug metabolism is expected to be limited (Rendic and Di Carlo, 1997). CYP2A6 exhibits relatively large variability among individuals owed to genetic polymorphisms (López-Flores et al., 2017).

Xanthine oxidase (XO) is an interconvertible form of the enzyme xanthine oxidoreductase and it is present in the cytoplasm and on the outer surface of the cell membrane (Harrison, 2002). It is the rate-limiting enzyme in purine catabolism and can oxidize a variety of endogenous substrates such as aldehydes, purines, pyrimidines and pteridines; in addition, it catabolizes aminopurines, such as 2-aminopurine, heterocyclic compounds, such as 4-hydroxypyrimidine, retinol (Krenitsky et al., 1972; Battelli et al., 2014) and different xenobiotics, such as antiviral and anticancer agents, thus, contributing to liver detoxification (Pritsos, 2000; Battelli et al., 2014).

N-acetyltransferase-2 (NAT2) is one of the two closely related isoforms of the xenobiotic-metabolizing enzymes arylamine N-acetyltransferases involved in the N-acetylation of arylamines and the O-acetylation of N-hydroxylated heterocyclic amines (Dupret and Rodrigues-Lima, 2005). It is a cytosolic conjugating enzyme expressed mainly in the liver and gut (Sim et al., 2012) and it participates in the acetylation of several prescribed drugs, such as sulfamethazine, sulfapyridine, procainamide, dapsone, nitrazepam, hydralazine, clonazepam, and isoniazid (Evans, 1989) and in the metabolism of environmental carcinogens including aromatic and heterocyclic amines (Dupret and Rodrigues-Lima, 2005). It is a polymorphic enzyme with 107 alleles arising from the combination of 43 point mutations; these polymorphisms separate individuals into slow or rapid acetylators (Boukouvala and Fakis, 2005; Hein et al., 2018).

Caffeine is an almost universal component of the human diet either as a natural constituent or as a common additive in various food products. It is a drug with large consumption among humans, it is easily accessible, well-tolerated and is characterized by excellent oral bioavailability (Nehlig, 2018). More than 80% of caffeine is metabolized by CYP1A2 to 1,7-dimethylxanthine (17X) which is bio-transformed to 1,7-dimethyluric acid (17U) by CYP2A6 and to 1-methylxanthine (1X) by CYP1A2. 1X is subsequently converted to 1-methyluric acid (1U) by XO. A small part of 1,7-dimethylxanthine is metabolized to 5-acetylamino-6-formylamino-3-methyluracil (AFMU) by NAT2 (Fig. 2) (Gu et al., 1992; Kot and Daniel, 2008). Therefore, the overall metabolism of caffeine is mediated mostly by CYP1A2, CYP2A6, XO and NAT2. Subsequently, caffeine metabolic ratios determined in saliva and urine have long been used as safe and non-invasive methods for the simultaneous assessment of the *in vivo* activity of CYP1A2, CYP2A6, XO and NAT2 (Asprodini et al., 1998; Begas et al., 2007; Hakooz, 2009).

While drugs remain the cornerstone of medicine, the use of herbal products and supplements as a source of healthcare, either as alternatives or as complementary to approved medications, has increased tremendously in the Western world over the past few years (Bodeker et al., 2005; Bandaranayake, 2006; Ekor, 2014). Unlike prescription drugs, however, over-the-counter herbal products are often used without prior proof of safety or efficacy introducing considerable risk for adverse herbal-drug interactions, especially when administered with drugs with narrow therapeutic index (Zhou et al., 2003; Bent, 2008; Zhang et al., 2015). Herbal-drug interactions may involve altered pharmacokinetic properties of administered drugs through inhibition or induction of metabolic enzymes mediated by various natural phytochemicals contained in herbs (Zhou et al., 2003; Wanwimolruk and Prachayasittikul, 2014). A common example is hyperforin, the active ingredient in St. John's wort, which promotes the expression of CYP3A4 in the small intestine and liver through interaction with pregnane xenobiotic receptor (PXR) (Moore et al., 2000) ultimately leading to altered pharmacokinetics of many drugs (Awortwe et al., 2019) and

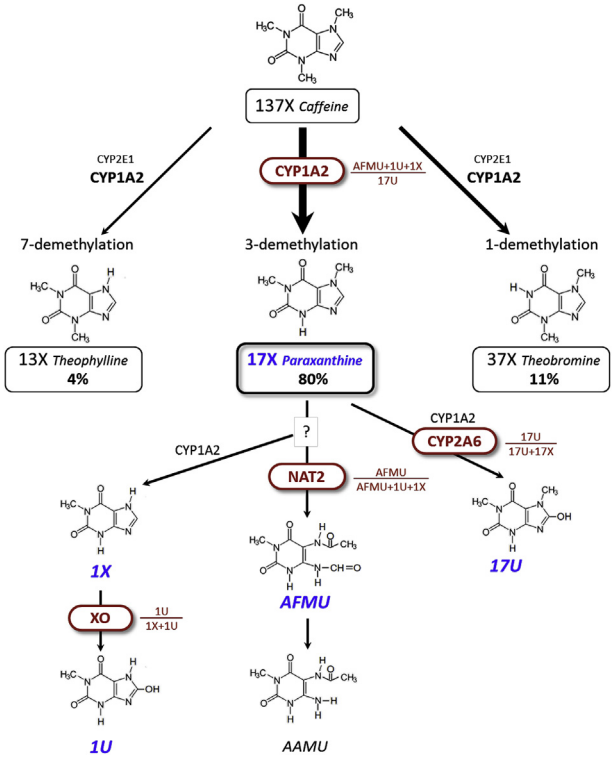


**Fig. 2. Caffeine metabolism pathways in human liver.** Most of caffeine is N-3 demethylated by CYP1A2 to paraxanthine (1,7-dimethylxanthine, 17X); this pathway accounts for about 80% of caffeine metabolism (heavy arrow; Lelo et al., 1986; Gu et al., 1992). N-1 demethylation to theobromine (3,7-dimethylxanthine, 37X) and N-7 demethylation to theophylline (1,3-dimethylxanthine, 13X) are also catalyzed by CYP1A2 and account for 11 and 4% of caffeine metabolism, respectively. Paraxanthine is oxidized to 1,7-dimethyluric acid (17U) by CYP2A6 and is demethylated to 1-methylxanthine (1X) which is subsequently oxidized to 1-methyluric acid (1U) by XO. NAT2 catalyzes the C8–N9 bond scission and the acetylation of paraxanthine to 5-acetylamino-6-formylamino-3-methyluracil (AFMU) which is then converted non-enzymatically into 5-acetylamino-6-amino-3-methyluracil (AAMU) in urine. Enzymes and metabolic molar ratios used as indices of enzyme activities are shown in red. Caffeine metabolites participating in the calculation of metabolic molar ratios are shown in blue. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

subsequent herb-drug interactions (Hammerness et al., 2003). Knowledge of possible effects of herbs on xenobiotic metabolizing enzymes is, therefore, necessary for assessing and minimizing clinical risks related to drug-herb interactions.

Despite the wealth of clinical data on the effects of *C. sativus* on depression, anxiety and other mental disorders (Lopresti and Drummond, 2014; Christodoulou et al., 2015; Shafiee et al., 2018; Tóth et al., 2019), diabetes and cardiovascular risk factors (Hausenblas et al., 2015; Pourmasoumi et al., 2019) or sexual dysfunction (Leone et al., 2018) in the literature, no study has to date addressed possible interactions of saffron with xenobiotic metabolism enzymes in humans. In fact, data of the effect of saffron constituents on enzyme activity is limited to an experimental animal study showing significant modulation of the activity of CYP3A, CYP2C11, CYP2B, and CYP2A in rat liver microsomes (Dovrtělová et al., 2015). Therefore, the purpose of the present study was to examine the effect of *C. sativus* on the *in vivo* activity of the xenobiotic metabolizing enzymes CYP1A2, CYP2A6, XO, and NAT2 in healthy volunteers using caffeine as a probe-drug.

## 2. Materials & methods

### 2.1. Subjects

Thirty-four non-smoking volunteers (twenty females and fourteen males) with mean age 38.79 ± 14.58 years (range 19–62), mean weight 70.63 ± 16.34 kg (range 52–130), mean height 1.70 ± 0.10m (range 1.57–1.90) and mean BMI 24.09 ± 3.66 (range 18.65–37.98) participated in the present study. Their health status was based on medical history, physical examination and recent routine blood tests. Volunteers did not have any history of medical illnesses (such as heart disease, inflammatory of autoimmune disease, or any other endocrine function deficiency) and were not receiving any medication, or taking vitamins, that could interfere with the study for at least seven days prior to and during the study period.

The protocol of the study was approved by the University Hospital, Larissa (Greece) Ethics Committee (No. 42396), was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) and adhered to the declaration of Helsinki guidelines. The participants were informed of every detail of the scope of the study and their written consent was obtained upon entering the study.

### 2.2. Study design

The study was an open-label, single-treatment, before-and-after trial in which each subject served as their own control. The overall study design is presented in Fig. 3. The study period lasted 17 days. CYP1A2, CYP2A6, XO and NAT2 phenotyping was carried out on days 1 (uncontrolled diet), 8 (before *C. sativus* intake) and 15 (after *C. sativus* intake) following a 36h abstinence from methylxanthine-containing foods and beverages (8:00 p.m. two days prior to the test until 8:00 a.m.

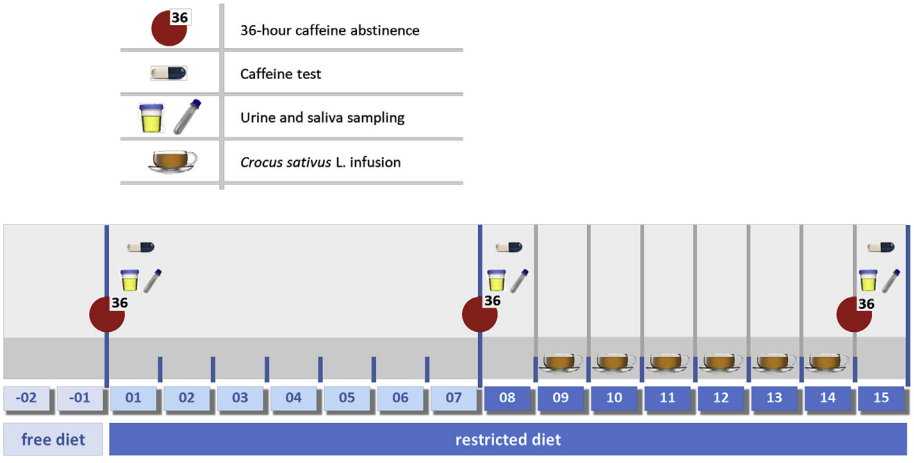


**Fig. 3. Schematic diagram of the experimental protocol followed in the study.** Saliva and urine samples were collected 6h after the caffeine test at days 1, 8 and 15 following 36h abstinence from caffeine-containing foods and beverages (filled circle designated with "36"). Enzyme metabolite ratios were determined following free diet (day 1), before (day 8) and after *C. sativus* (day 15) infusion intake (300 mg in 150 mL hot water, days 9–14). Samples at days 8 and 15 were collected under restricted diet.

**Table 1**
Metabolic ratios of CYP1A2, CYP2A6, XO and NAT2 determined before and after *C. sativus* infusion for 6 days under restricted diet.

| ID | Sex | CYP1A2 | | | | | | CYP2A6 | | | XO | | | NAT2 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | saliva | | | urine | | | urine | | | urine | | | urine "slow" | | | urine "fast" | | |
| | | free | before | after | free | before | after | free | before | after | free | before | after | free | before | after | free | before | after |
| 1 | F | 0.37 | 0.32 | 0.30 | 3.18 | 3.24 | 2.89 | 0.51 | 0.36 | 0.44 | 0.51 | 0.50 | 0.49 | 0.10 | 0.09 | 0.10 | | | |
| 2 | F | 0.21 | 0.16 | 0.27 | 1.59 | 1.32 | 1.90 | 0.58 | 0.67 | 0.57 | 0.54 | 0.42 | 0.53 | | | | 0.36 | 0.35 | 0.42 |
| 3 | F | 0.28 | 0.34 | 0.30 | 2.52 | 3.62 | 3.43 | 0.43 | 0.28 | 0.29 | 0.51 | 0.46 | 0.35 | 0.08 | 0.09 | 0.08 | | | |
| 4 | F | | | | 4.90 | 4.70 | 5.53 | 0.29 | 0.36 | 0.58 | 0.58 | 0.64 | 0.63 | | | | 0.35 | 0.40 | 0.38 |
| 5 | F | 0.33 | 0.45 | 0.48 | 2.72 | 3.59 | 2.54 | 0.62 | 0.35 | 0.42 | 0.63 | 0.58 | 0.59 | | | | 0.54 | 0.53 | 0.48 |
| 6 | F | 0.26 | 0.23 | 0.24 | 3.71 | 2.51 | 2.54 | 0.38 | 0.38 | 0.45 | 0.39 | 0.49 | 0.43 | | | | 0.40 | 0.37 | 0.33 |
| 7 | F | 0.43 | 0.50 | 0.38 | 3.27 | 4.17 | 2.88 | 0.36 | 0.49 | 0.47 | 0.54 | 0.49 | 0.54 | 0.11 | 0.12 | 0.10 | | | |
| 8 | F | 0.33 | 0.29 | 0.29 | 3.48 | 3.29 | 3.49 | 0.61 | 0.68 | 0.66 | 0.57 | 0.57 | 0.56 | 0.10 | 0.09 | 0.08 | | | |
| 9 | F | 0.42 | 0.43 | 0.44 | 3.04 | 2.80 | 3.29 | 0.78 | 0.71 | 0.57 | 0.60 | 0.51 | 0.58 | 0.08 | 0.06 | 0.09 | | | |
| 10 | F | | | | 2.09 | 2.14 | 2.59 | 0.49 | 0.59 | 0.58 | 0.54 | 0.57 | 0.57 | 0.09 | 0.08 | 0.08 | | | |
| 11 | F | 0.50 | 0.56 | 0.42 | 3.47 | 4.59 | 3.72 | 0.31 | 0.39 | 0.52 | 0.51 | 0.50 | 0.52 | 0.06 | 0.05 | 0.07 | | | |
| 12 | F | 0.39 | 0.29 | 0.27 | 3.50 | 3.16 | 3.16 | 0.46 | 0.58 | 0.55 | 0.51 | 0.56 | 0.57 | 0.07 | 0.05 | 0.06 | | | |
| 13 | F | 0.41 | 0.50 | 0.32 | 3.38 | 3.04 | 2.85 | 0.65 | 0.70 | 0.62 | 0.57 | 0.59 | 0.57 | | | | 0.52 | 0.48 | 0.53 |
| 14 | F | | | | 3.28 | 3.19 | 3.06 | 0.39 | 0.59 | 0.65 | 0.56 | 0.54 | 0.55 | 0.10 | 0.06 | 0.10 | | | |
| 15 | F | | | | 2.99 | 2.55 | 2.72 | 0.38 | 0.74 | 0.76 | 0.50 | 0.53 | 0.55 | | | | 0.47 | 0.45 | 0.36 |
| 16 | F | 0.28 | 0.34 | 0.50 | 3.35 | 2.98 | 4.40 | 0.51 | 0.48 | 0.62 | 0.53 | 0.60 | 0.61 | 0.12 | 0.09 | 0.10 | | | |
| 17 | F | 0.51 | 0.53 | 0.58 | 3.21 | 2.89 | 2.64 | 0.63 | 0.52 | 0.73 | 0.59 | 0.64 | 0.61 | 0.06 | 0.08 | 0.07 | | | |
| 18 | F | 0.47 | 0.44 | 0.43 | 4.42 | 3.86 | 3.65 | 0.40 | 0.46 | 0.68 | 0.63 | 0.59 | 0.63 | 0.10 | 0.06 | 0.10 | | | |
| 19 | F | 0.50 | 0.26 | 0.35 | 4.62 | 3.19 | 3.23 | 0.44 | 0.34 | 0.37 | 0.50 | 0.50 | 0.52 | 0.09 | 0.09 | 0.08 | | | |
| 20 | F | 0.54 | 0.58 | 0.49 | 4.78 | 5.37 | 4.71 | 0.63 | 0.56 | 0.63 | 0.59 | 0.59 | 0.57 | 0.09 | 0.09 | 0.09 | | | |
| 21 | M | 0.81 | 0.64 | 0.46 | 5.54 | 5.37 | 5.42 | 0.42 | 0.45 | 0.58 | 0.56 | 0.51 | 0.58 | | | | 0.50 | 0.41 | 0.36 |
| 22 | M | 0.62 | 0.71 | 0.53 | 4.07 | 3.79 | 3.41 | 0.77 | 0.74 | 0.71 | 0.61 | 0.63 | 0.60 | | | | 0.32 | 0.33 | 0.36 |
| 23 | M | 0.46 | 0.65 | 0.61 | 3.92 | 4.97 | 4.42 | 0.52 | 0.80 | 0.59 | 0.46 | 0.48 | 0.44 | 0.16 | 0.17 | 0.13 | | | |
| 24 | M | 0.28 | 0.25 | 0.24 | 3.80 | 3.32 | 3.23 | 0.58 | 0.76 | 0.64 | 0.61 | 0.67 | 0.64 | | | | 0.42 | 0.39 | 0.34 |
| 25 | M | 0.63 | 0.72 | 0.53 | 4.11 | 3.91 | 3.27 | 0.68 | 0.74 | 0.74 | 0.48 | 0.55 | 0.54 | 0.07 | 0.09 | 0.09 | | | |
| 26 | M | 0.69 | 0.63 | 0.43 | 6.09 | 4.99 | 3.84 | 0.30 | 0.62 | 0.68 | 0.49 | 0.47 | 0.47 | 0.10 | 0.12 | 0.12 | | | |
| 27 | M | 0.64 | 0.70 | 0.52 | 4.56 | 4.16 | 3.64 | 0.65 | 0.64 | 0.74 | 0.64 | 0.62 | 0.65 | | | | 0.32 | 0.42 | 0.38 |
| 28 | M | 0.35 | 0.47 | 0.41 | 3.33 | 3.29 | 3.74 | 0.65 | 0.61 | 0.38 | 0.55 | 0.57 | 0.57 | | | | 0.47 | 0.50 | 0.53 |
| 29 | M | 1.26 | 0.99 | 0.78 | 7.25 | 5.72 | 5.56 | 0.45 | 0.54 | 0.78 | 0.49 | 0.52 | 0.56 | | | | 0.37 | 0.35 | 0.42 |
| 30 | M | 0.71 | 0.57 | 0.59 | 4.37 | 4.08 | 4.02 | 0.52 | 0.63 | 0.65 | 0.46 | 0.51 | 0.54 | 0.09 | 0.09 | 0.08 | | | |
| 31 | M | 0.44 | 0.39 | 0.30 | 4.21 | 3.61 | 2.97 | 0.48 | 0.58 | 0.45 | 0.63 | 0.60 | 0.62 | | | | 0.26 | 0.27 | 0.26 |
| 32 | M | 0.67 | 0.99 | 0.69 | 4.82 | 7.70 | 5.48 | 0.44 | 0.39 | 0.51 | 0.53 | 0.55 | 0.58 | | | | 0.29 | 0.24 | 0.30 |
| 33 | M | 0.48 | 0.48 | 0.33 | 4.87 | 3.70 | 3.87 | 0.78 | 0.88 | 0.67 | 0.50 | 0.54 | 0.52 | 0.12 | 0.09 | 0.09 | | | |
| 34 | M | 1.49 | 0.94 | 0.66 | 5.56 | 4.87 | 3.50 | 0.59 | 0.59 | 0.50 | 0.58 | 0.57 | 0.54 | 0.11 | 0.10 | 0.10 | | | |
| | Mean | 0.53 | 0.51 | 0.44* | 3.94 | 3.81 | 3.58 | 0.52 | 0.56 | 0.58 | 0.54 | 0.55 | 0.55 | 0.09 | 0.09 | 0.09 | 0.40 | 0.39 | 0.39 |
| | SD | 0.28 | 0.22 | 0.14 | 1.15 | 1.20 | 0.92 | 0.14 | 0.15 | 0.12 | 0.06 | 0.06 | 0.06 | 0.02 | 0.03 | 0.02 | 0.09 | 0.08 | 0.08 |

*p = 0.002 *vs.* before *C. sativus* values (paired-samples *t*-test).

of the day of the test; Fig. 3). In the morning of the test days, the subjects followed the caffeine protocol: after voiding their bladders they consumed a 200 mg caffeine-containing capsule and 6 h later, spot samples of unstimulated saliva and urine were collected. Urine samples were acidified to pH 3.5 to ensure the stability of AFMU (Wong et al., 2002). Saliva samples (1–2 mL) and 1 mL urine aliquots were number-coded and were stored at −20 °C until analysis. No blank urine or saliva samples were provided prior to the caffeine test.

In the morning of day 1 the participants carried out the caffeine test and they commenced a restricted diet which lasted throughout the rest of the study. Participants were asked to consume a controlled carotenoid-free diet (foods excluded: tomatoes, peppers, carrots, zucchinis, spinach, lettuce, peas, cruciferous vegetables, celery, asparagus, beetroots, pomegranates, apricots, peaches, melons, watermelons, oranges, tangerines, grape-fruit juice, apples, onions, cherries) for 14 days; during this period, they were also asked to abstain from alcohol, herbs, spices and charcoal-broiled meat. They were allowed to consume red meat, chicken, fish (except red fish such as salmon), fats and oils, egg-white, cereals, milk and dairy products, white bread, pasta, rice and potatoes, all boiled or cooked in low temperatures. In the evening of day 8 the volunteers prepared the first *C. sativus* infusion. The intervention period began in the morning of day 9 when the participants consumed the *C. sativus* infusion and they continued to receive it for the next 5 days. Participants were asked to report possible adverse events such as headache, nausea, dizziness, fainting, cough, dyspnea, palpitations, fatigue, dysuria, altered appetite and gastrointestinal disturbances related to the intervention.

### 2.3. *C. sativus* infusion preparation

*C. sativus* powder (300 mg) was added in ~150 mL tap water heated at 70°-80 °C, stirred and left to stand overnight in dark. The following morning, the solution was stirred and consumed by the volunteers. Organic *C. sativus* powder was purchased from the local market of Larissa, Greece. The product is distributed under the trade mark "Krokos Kozanis" Kozani, Greece and is produced by the "Kozani Saffron Producers Cooperative" which has developed a certified organic cultivation of saffron and has gained quality assurance certificate ISO 9001 in 1997. The name "Krokos Kozanis" is registered as Protected Designation of Origin (PDO) (www.safran.gr).

### 2.4. Chemicals

Caffeine (1,3,7-trimethylxanthine-137X) was purchased from Merck (Germany); caffeine metabolites 1,7-methylxanthine (17X), 1,7-dimethyluric acid (17U) and 1-methyluric acid (1U) were purchased from Sigma (Germany). 1-methylxanthine (1X) was purchased by TCI (Belgium). 5-acetylamino-6-formylamino-3-methyluracil (AFMU) was kindly provided by Professor Wolfgang Pfleiderer (Konstanz University). Methanol and acetonitrile were of HPLC grade; acetic acid, chloroform, isopropanol and ammonium sulfate were of analytical grade.

### 2.5. Analysis of caffeine and caffeine metabolites in urine and saliva samples

High performance liquid chromatography (HPLC) was used for the analysis of caffeine metabolites in urine (Begas et al., 2007) and caffeine (1,3,7-trimethylxanthine, 137X) and 17X in saliva (Begas et al., 2015) as previously described. In all analyses separation was achieved using a Kromasil 100 C18 column (5 μm, 250 × 4.6 mm i.d.; Macherey-Nagel, Germany) operated at 30 °C.

Caffeine metabolites were extracted from 200 μL urine samples using 6 mL chlorophorm-isopropanol solution (85/15, v/v); the mobile phase (0.1% acetic acid/ methanol/acenonitrile 92/4/5, v/v) was delivered at 0.7 mL/min for 5min and at 1.1 mL/min from 5 to 20min and detection was achieved at 280 nm. Inter-day coefficients of variation for AFMU, 1U, 1X, 17U and 17X ranged between 3.34-9.00% and 2.04–5.37% for concentrations 30 and 300μM, respectively. Bias for the five metabolites ranged between 0.14 and 3.40% and −2.70-0.71% for concentrations 30 and 300μM, respectively (n = 8).

Caffeine and 17X were extracted from 200 μL saliva samples using 4 mL chlorophorm-isopropanol solution (85/15, v/v); the mobile phase (0.1% acetic acid/methanol/acetonitrile 80/20/2, v/v) was delivered at 1.0 mL/min and detection was achieved at 273 nm. Twelve saliva samples were destroyed due to facility power failure and were, consequently, discarded (missing values, Table 1). Inter-day coefficients of variation for 17X were 4.35 and 5.40% for concentrations 1.50 and 15.00μM, respectively; the respective biases were 4.74 and 0.36% (n = 8). Inter-day coefficients of variation for 137X were 4.00 and 4.60% for concentrations 1.50 and 15.00μM, respectively; the respective biases were 4.46 and −2.07% (n = 8).

### 2.6. Assessment of metabolite ratios

CYP1A2 activity was estimated by the caffeine metabolite molar concentration ratio (AFMU + 1U + 1X)/17U in spot urine samples (Campbell et al., 1987; Begas et al., 2007) and by the caffeine metabolite ratio 17X/137X in saliva samples (Fuhr and Rost, 1994; Begas et al., 2015). CYP2A6, XO and NAT2 activities were estimated using the phenotypic indices determined by the urinary molar concentrations 17U/(17U + 17X) (Begas et al., 2017; Grant et al., 1983), 1U/(1U + 1X) (Kalow and Tang, 1991) and AFMU/(AFMU + 1U + 1X) (Rostami-Hodjegan et al., 1996), respectively.

### 2.7. Statistical analysis

All data are presented as mean ± standard deviation (SD). The two-tailed, paired-samples, Student t-test was used to examine differences in metabolite ratios between treatments. P values $< 0.05$ were considered statistically significant. Statistical analyses were performed using SPSS v24 software (IBM, USA).

## 3. Results

All 34 subjects completed the treatment period with no report of any adverse effects suggesting good tolerability of the *C. sativus* infusion intake. The metabolite ratios calculated for all volunteers reflecting the activity of CYP1A2, CYP2A6, XO, and NAT2 at baseline (free diet) and before and after *C. sativus* infusion intake under restricted diet, are presented in Table 1 and Figs. 4 and 5.

CYP1A2 metabolite ratios in saliva exhibited a statistically significant 13.7% reduction (range −36.0 to +68.8) following *C. sativus* infusion ingestion (before: 0.51 ± 0.22, after: 0.44 ± 0.14; p = 0.002, Fig. 4A); the metabolite ratios in saliva decreased in twenty-one, increased in eight and remained the same in one subject. The reduction could be attributed to the male rather than the female volunteers because when women and men were considered separately, a significant reduction (21.5%) in CYP1A2 metabolite ratios was observed in male (before: 0.65 ± 0.22, after: 0.51 ± 0.16; p = 0.0001), but not female (before: 0.39 ± 0.13, after: 0.38 ± 0.10; p = 0.67) volunteers (Table 2; Fig. 4A). This sex-related dimorphism is reflected in the different proportion between men (~90%) and women (~50%) exhibiting reduced CYP1A2 metabolite ratios following *C. sativus* infusion ingestion (Fig. 4C).

CYP1A2 metabolite ratios in urine exhibited a minor 6.0% non-significant decrease (range −30.9 to +47.0) was observed in CYP1A2 metabolite ratios following *C. sativus* infusion intake (before: 3.81 ± 1.20, after: 3.58 ± 0.92; p = 0.054, Fig. 4B); the metabolite ratios in urine decreased in twenty-one and increased in thirteen subjects. As in saliva samples, when women and men were considered separately, CYP1A2 metabolite ratios exhibited statistically significant reduction (11.04%) in male (before: 4.53 ± 1.19, after: 4.03 ± 0.87; p = 0.017), but not female (before: 3.31 ± 0.93, after: 3.26 ± 0.84; p = 0.74) volunteers (Table 2; Fig. 4B).

In subjects 5, 17, 21, 28, 30 and 33 CYP1A2 metabolite ratios shifted towards the opposite direction between saliva and urine samples following *C. sativus* infusion intake.

CYP1A2 metabolite ratios were not altered when volunteers changed from a customary home diet to the restricted diet both in saliva and in urine samples (saliva: free diet 0.53 ± 0.28 *vs* restricted diet 0.51 ± 0.22, p = 0.63; urine: free diet 3.94 ± 1.15 *vs* restricted diet 3.81 ± 1.20, p = 0.40).

It should be noted that CYP1A2 metabolite ratios were higher in male compared to female volunteers under all treatments in both saliva and urine samples (Table 2).

The urine metabolite ratios reflecting CYP2A6 (before: 0.56 ± 0.15, after: 0.58 ± 0.12; p = 0.43, Fig. 5A) and XO (before: 0.55 ± 0.06, after: 0.55 ± 0.06; p = 0.49, Fig. 5B) activity remained unaltered following *C. sativus* infusion intake.

The activity of NAT2 is primarily genetically determined exhibiting bimodal distribution among different ethnic groups (Sim et al., 2012). Therefore, possible alterations in NAT2 activity were examined separately in slow- and rapid-acetylator phenotypes; phenotypes were discriminated based on the cut-off value 0.25 which we have previously shown to separate slow-from rapid acetylators within the Greek population (Begas et al., 2007). Based on this cut-off value, twenty subjects were classified as slow- and fourteen as rapid-acetylators. The ingestion of C. *sativus* infusion had no effect upon slow- or fast-acetylators (slow-acetylators, before: 0.09 ± 0.02, after: 0.09 ± 0.03, p > 0.05; fast-acetylators, before: 0.39 ± 0.08, after: 0.39 ± 0.08, p > 0.05; Fig. 5C).

## 4. Discussion

Herbal products may alter the activity of xenobiotic metabolizing enzymes leading to potential pharmacokinetic herb-drug interactions and, subsequently, to important clinical consequences. Given the growing interest in the medicinal uses of *C. sativus*, its potential influence upon drug metabolizing enzymes becomes an important issue. The present study is the first to demonstrate that six-day consumption of *C. sativus* infusion decreases CYP1A2, but not CYP2A6, XO or NAT2, metabolic ratios in saliva and urine samples indicating reduction in CYP1A2 *in vivo* activity in healthy volunteers. The reduction in CYP1A2 metabolic ratio is attributed to decreased CYP1A2 activity in men, rather than in women, and it is more prominent in saliva samples.

Only a few studies have reported the effects of herbal products on CYP1A2, CYP2A6, XO or NAT2 activity in humans using caffeine as a metabolic probe. Significant reduction in the activity of CYP1A2 has been reported for quercetin (Chen et al., 2009), curcumin (Chen et al., 2010), genistein (Chen et al., 2011), Angelicae dahuricae radix (Yi et al., 2009) and Keishi-bukuryo-gan (Saruwatari et al., 2012). Conversely, no effect on CYP1A2 activity has been reported for garlic oil, Ginkgo biloba, Panax ginseng (Gurley et al., 2002, 2005), peppermint tea (Begas et al., 2017), or Sideritis scardica (Begas et al., 2018). The

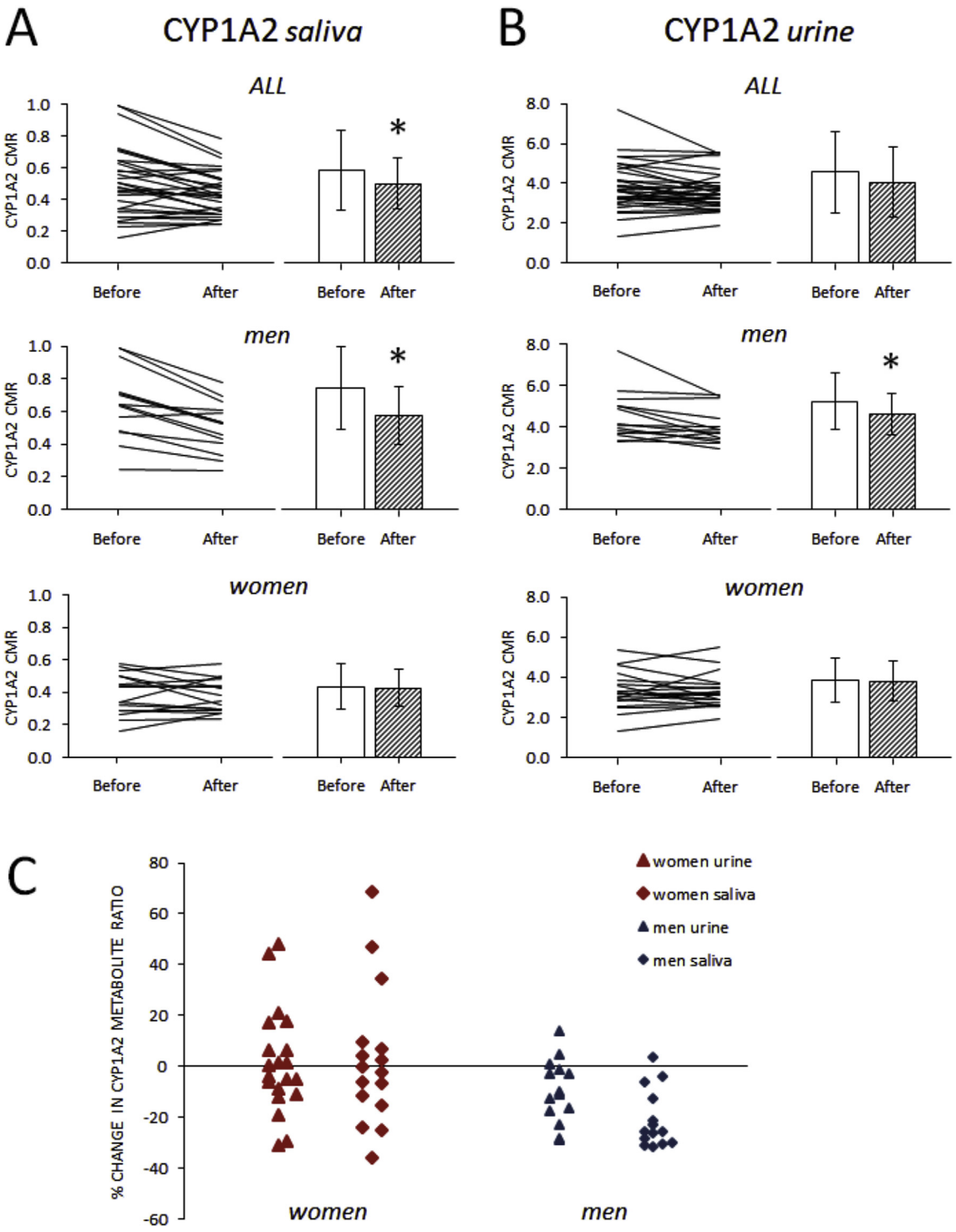


**Fig. 4. Effect of C. *sativus* on CYP1A2 activity.** Metabolite ratios were estimated during the restricted diet before and after *C. sativus* infusion consumption. **A.** CYP1A2 index 17X/137X determined in saliva samples; individual data (left) and mean values ± S.D. (right). **B.** CYP1A2 index (AFMU + 1U + 1X)/17U determined in urine samples; individual data (left) and mean values ± S.D. (right). **C.** Column chart of percentage of increase (positive values) or reduction (negative values) of CYP1A2 index determined in saliva and urine samples of female and male volunteers.

effect of St John's wort is disputed as both no effect (Wang et al., 2001; Gurley et al., 2005) and increased CYP1A2 activity have been reported (Gurley et al., 2002; Wenk et al., 2004). The activity of CYP2A6 is increased by quercetin (Chen et al., 2009), curcumin (Chen et al., 2010) and genistein (Chen et al., 2011), is decreased in men by Sideritis scardica (Begas et al., 2018), whereas Keishi-bukuryo-gan (Saruwatari et al., 2012) and peppermint tea (Begas et al., 2017) produce no effect. XO activity is increased by quercetin (Chen et al., 2009), decreased by genistein (Chen et al., 2011) and is not affected by St John's wort (Wenk et al., 2004), curcumin (Chen et al., 2010), Keishi-bukuryo-gan (Saruwatari et al., 2012), peppermint tea (Begas et al., 2017) and Sideritis scardica (Begas et al., 2018). No effect has been reported for NAT2 by St John's wort (Wenk et al., 2004), curcumin (Chen et al., 2010), genistein (Chen et al., 2011), Keishi-bukuryo-gan (Saruwatari et al., 2012) and Sideritis scardica (Begas et al., 2018) with the exception of peppermint tea which reduces its activity (Begas et al.,

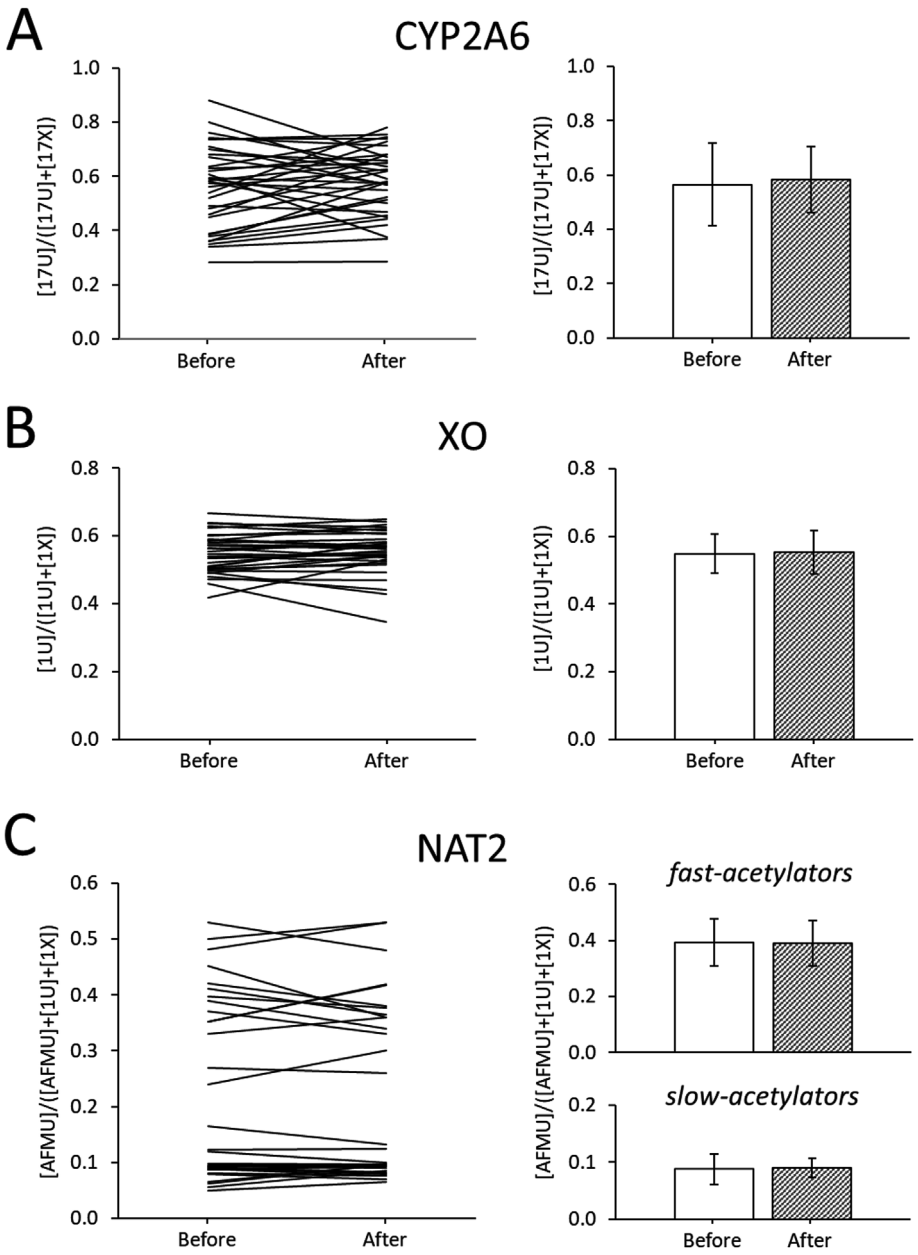


**Fig. 5. Effect of C. *sativus* on CYP2A6, XO and NAT2 activity.** All metabolite ratios were estimated in urine during the restricted diet before and after C. *sativus* infusion intake. In all panels individual data are shown in left and mean values ± S.D. are shown in right. **A.** CYP2A6 index: 17U/(17U + 17X). **B.** XO index: 1U/(1U + 1X). **C.** NAT2 index: AFMU/(AFMU + 1U + 1X).

**Table 2**
Differences in CYP1A2 metabolite ratios between men and women under various diet conditions.

| | men | women | p |
|---|---|---|---|
| ***saliva*** | | | |
| free diet | 0.68 ± 0.33 | 0.39 ± 0.10 | 0.0023 |
| restricted diet | 0.65 ± 0.22 | 0.39 ± 0.13 | 0.0003 |
| restricted diet + *C. sativus* | 0.51 ± 0.16 | 0.38 ± 0.10 | 0.0120 |
| ***urine*** | | | |
| free diet | 4.75 ± 1.05 | 3.37 ± 0.84 | 0.0002 |
| restricted diet | 4.53 ± 1.19 | 3.31 ± 0.93 | 0.0020 |
| restricted diet + *C. sativus* | 4.03 ± 0.87 | 3.26 ± 0.84 | 0.0151 |

2017). Conversely, quercetin induces NAT2 activity by almost 89% (Chen et al., 2009). The overall data suggest that different herbal products exert diverse effects on the activity of specific enzymes.

The differential effect of herbal products, between men and women, on CYP activity has been previously reported both in human and in animal studies. Consumption of St John's Wort for 28 days elicits 74% greater induction of CYP3A4 in women compared to men (Gurley et al., 2002). CYP2A6 activity is significantly reduced in men, but not women, following consumption of *Sideritis scardica* decoction for a week (Begas et al., 2018). Long-term treatment with tea polyphenols increases P450 content specifically in male rats (Liu et al., 2003), while the flavonoid quercetin inhibits the activity of CYP2E1 *in vitro* only in microsomes from male pigs (Ekstrand et al., 2015).

Although the source of the apparent differential effect of *C. sativus* on CYP1A2 activity between male and female volunteers has not been addressed in the present study, such a differential effect may be explained in light of the sex-related dimorphism in several physiological and molecular parameters concerning pharmacotherapy. First, women, compared to men, are characterized by different physiological factors

such as gastric acidity, intestinal motility, body weight and composition, organ size, blood volume and renal excretion, which may potentially influence pharmacokinetic parameters of administered substances (Marazziti et al., 2013; Islam et al., 2017). Second, sex-related dimorphism in molecular factors involved in drug elimination, such as drug-metabolizing enzymes and drug transporters, may result in differential drug disposition between men and women (Meibohm et al., 2002). For example, olanzapine, an antipsychotic drug metabolized by CYP1A2, reaches higher plasma concentrations (Kelly et al., 1999, 2006) and is cleared 38% slower (Bigos et al., 2008) in women compared to men. These findings could be attributed, at least in part, to sex-related differences in endogenous basal CYP1A2 activity. Indeed, it is widely accepted that CYP1A2 activity is higher in men compared to women (Relling et al., 1992; Bock et al., 1994; Shimada et al., 1994; Kashuba et al., 1998; Tantcheva-Poór et al., 1999; Ou-Yang et al., 2000). The data in the present study support these literature findings as women exhibited lower CYP1A2 activity under all diet conditions (Table 2). Furthermore, saffron exerted an intriguingly different effect pattern on CYP1A2 activity between men and women; whereas it reduced CYP1A2 activity in almost all male subjects, the direction of the effect in women was unpredictable (Fig. 4C). This finding could be possibly explained by the fluctuation of CYP1A2 activity across menstrual cycle. Indeed, the activity of CYP1A2 reaches its lowest level during the late follicular phase (Nagata et al., 1997; Kamimori et al., 1999; Asprodini et al., 2019), an effect which coincides temporally with the highest serum caffeine concentration (Schliep et al., 2016). Since menstrual cycle was not accounted for in the present study, it is possible that the phase of the menstrual cycle, that a female volunteer was at the time of sample collection, influenced the direction of the effect of saffron on CYP1A2 activity.

In addition to sex, BMI has been considered as an independent factor that might influence the activity of xenobiotic metabolizing enzymes. A small negative effect of BMI on CYP1A2-dependent caffeine clearance has been observed in humans (Tantcheva-Poór et al., 1999), although in other studies no such effect has been reported (Le Marchand et al., 1997; Chiney et al., 2011). Previous studies have suggested that elevated BMI is related with higher NAT2 (Chiney et al., 2011) and XO (Klisic et al., 2018) activities in humans and higher CYP2A5 (CYP2A6 human ortholog) activity in mice (Tomankova et al., 2015). Nevertheless, since the design of the present study was a before-and-after trial where each subject served as their own control, the BMI of each volunteer might influence their baseline enzyme activity rather than the outcome of C. *sativus* on the activity of the enzymes studied.

Interestingly, although a statistically significant reduction was observed in male volunteers both in saliva and urine samples, when both men and women were considered in the analysis the activity of CYP1A2 was significantly reduced only in saliva samples. These data indicate that saliva caffeine metabolite ratios exhibit, in relation to urinary ratios, higher sensitivity in discerning alterations in CYP1A2 activity. Indeed, the caffeine metabolite ratio paraxanthine/caffeine determined in saliva is considered the most advantageous index of CYP1A2 activity as it most closely resembles systemic caffeine clearance (Fuhr and Rost, 1994). Conversely, CYP1A2 metabolite ratios determined in urine are based on secondary or tertiary metabolites which are not formed exclusively by CYP1A2 and, additionally, may be influenced by urinary flow (Rostami-Hodjegan et al., 1996).

Like all herbal products, *C. sativus* is a complex mixture of more than one constituent. It is currently unknown which of the components of *C. sativus* are responsible for the reduced CYP1A2 activity in men. Due to the multitude of active ingredients contained in any herbal product, it is difficult to correlate specific ingredients with the impact that these might have on enzyme activity. This issue is further complicated by the currently unknown constituent bioavailability of orally administered *C. sativus* in humans. In fact, there is only one report in the literature showing crocetin accumulation in human serum following saffron consumption (Chryssanthi et al., 2011). Preclinical studies have suggested that non-absorbable hydrophilic components such as glycosides, like crocins, are biotransformed by β-glucosidases, located in the intestinal epithelial cells or in the microflora within the intestinal lumen, to their lipophilic aglycon product, in this case crocetin, before they are absorbed into the epithelial cells by passive diffusion (Asai et al., 2005; Xi et al., 2007; Zhang et al., 2017). Correspondingly, the low bioavailability of the glycoside picrocrocin (Kyriakoudi et al., 2015) is enhanced following conversion to its aglycon form, safranal, by digestive enzymes or by microbiota metabolism *in vivo* (Marín et al., 2015). Although one might argue that findings in animal studies may not be extrapolated to humans, it is possible that crocetin, rather than crocins, is the bioactive molecule following *C. sativus* oral ingestion, although the possibility of absorption of crocins or other active constituents such as safranal or kaempferol cannot be excluded (Karkoula et al., 2018).

It has been argued that the medicinal use of crude herbal extracts offers advantages over the use of purified ingredients owed to the synergistic interactions among its constituents (Williamson, 2001; Li, 2002); in fact, due to this synergy the ingredient concentrations of herbs needed to exert medicinal effects are very low (Bode and Dong, 2009). Tied to this holistic approach is the, potentially, ineffective correlation between individual ingredients of herbs and their biological effects, such as enzyme activity, as it would disregard the unique advantage of synergism among different herb components. Nevertheless, several *in vitro* and *in vivo* studies have examined the effects of individual *C. sativus* ingredients, or related molecules, on enzyme activity and are outlined below. Crocin has been demonstrated to downregulate the activity of CYP2B, CYP2C11, CYP3A4, CYP2E1 in rat liver microsomes (Dovrtělová et al., 2015) and the activity of CYP2E1 in rat kidney microsomes (Hassan et al., 2015). The lipophilic carotenoid lycopene, present in trace amounts in *C. sativus* (Melnyk et al., 2010), has been shown to inhibit CYP1A1 and CYPB1 activity *in vitro* (Wang and Leung, 2010), as well as CYP2E1, but not CYP1A2, activity in rat liver microsomes (Louisa et al., 2009). Indirect evidence suggests that lycopene inhibits CYP1A2 activity as it reduces the mutagenicity of PhIP, a heterocyclic amine which is metabolically activated by CYP1A2 (Weisburger et al., 1998). The evidence on the inhibitory effect of lycopene reported in experiments *in vitro* has not been corroborated, however, in animal studies *in vivo* as dietary supplementation of lycopene has no effect on rat CYP1A1 or CYP1A2 activities (Jewell and O'Brien, 1999). Overall, crocin and, the structurally analogous, lycopene inhibit the activity of several CYPs in rodents, although the effect on CYP1A2 activity remains elusive. The activity of XO has been reported to be reduced in the kidneys of diabetic rats treated orally with crocin (Altinoz et al., 2015) and in skeletal muscle after exhaustive exercise in rats treated with lycopene (Liu et al., 2005), whereas evidence on the effect of crocin or lycopene on CYP2A6 or NAT2 activity is lacking. Evidence in the literature addressing the effect of safranal, or its glycoside picrocrocin, on xenobiotic metabolizing enzymes is also lacking except from a report showing that safranal upregulates CYP2B, CYP2C11 and CYP3A activities in rat liver microsomes (Dovrtělová et al., 2015). Finally, kaempferol, a flavonoid which has been identified in the stigmata of Greek saffron (Tarantilis et al., 1995), has been shown to be a potent inhibitor of CYP1A2 (Shimada et al., 2010), XO (NAGAO et al., 1999) and, to a lesser extent, NAT2 (Kukongviriyapan et al., 2006) and CYP2A6 (Tiong et al., 2010) activities *in vitro*. However, the *in vivo* action of kaempferol has been questioned due to poor oral bioavailability and extensive metabolism (Zabela et al., 2016). In summary, the bioactive compounds contained in dietary saffron have little influence on CYP2A6, XO and NAT2 activities. The reduction in CYP1A2 activity observed in men in the present study may reflect the combined effect, inhibiting or synergistic, of all the ingredients of *C. sativus*.

A potential limitation of this study is the lack of phytochemical analysis to quantitate the active ingredients of the product used as constituent content may vary among geographical areas and, within the

same area, among batches which may impact on CYP1A2 activity. However, this potential limitation appears minimal as the chemical composition of *C. sativus*, originating from Kozani, Greece, has been thoroughly explored by various investigators and the analyses have yielded comparable results with respect to its major active constituents (Papandreou et al., 2006; García-Rodríguez et al., 2017; Karkoula et al., 2018), regardless of the unavoidable batch-to-batch variations. Another potential limitation is the short intervention period which would be insufficient in the clinical management of chronic diseases such as mental disorders (Tsolaki et al., 2016; Lopresti and Drummond, 2017). In case of longer treatment, it would be interesting to evaluate the timeline of CYP1A2 alteration as it has been reported that the effect that an herbal product exerts on enzyme activity may be dependent on intervention duration. For example, the inducing effect of St John's wort on CYP1A2 is observed after 28 (Uchida et al., 2006) but not after 8 or 14 (Wang et al., 2001) days of supplementation.

It has been suggested that herbal products may act as health-promoting, disease-preventing dietary supplements by reducing the activity of phase I carcinogen-activating enzymes and by enhancing phase II detoxifying enzymes (Moon et al., 2006; Wu et al., 2017). Since CYP1A2 participates in the metabolic activation of several environmental procarcinogens, such as aromatic and heterocyclic amines (Pelkonen et al., 2008), modulation of its activity is expected to affect cancer risk in individuals exposed to these procarcinogens. The reduction in CYP1A2 activity in men may potentially act as a protective mechanism against chemically induced cancer. Despite this positive piece of evidence, however, this result should be interpreted with caution as CYP1A2 activity is influenced by numerous factors (Koonrungsesomboon et al., 2018) and its evaluation in well-designed studies with a large cohort of subjects is required.

Numerous clinical studies have pointed out the potential therapeutic benefits of *C. sativus* in humans. For example, administration of saffron to patients suffering from mental disorders such as depression and Alzheimer's disease (Lopresti and Drummond, 2014; Tsolaki et al., 2016; Leone et al., 2018; Shafiee et al., 2018; Yang et al., 2018), retinal degeneration (Bisti et al., 2014; Lashay et al., 2016), cardiovascular disease (Kamalipour and Akhondzadeh, 2011; Hatziagapiou and Lambrou, 2018) or diabetes (Milajerdi et al., 2018; Yaribeygi et al., 2019) had a favorable disease outcome. Actually, clinical guidelines by the Canadian Network for Mood and Anxiety Treatments have recently recommended saffron as a third-line mono- or adjunctive-therapy for the treatment of mild to moderate Major Depressive Disorder (Ravindran et al., 2016). Despite the relative wealth of literature on the significant clinical effects of saffron in different diseases, there remains a dearth of information on interaction between conventional treatments and saffron. This is an important issue because even if drugs and herbals may be safe when taken alone, potential herb-drug interactions with important clinical consequences may arise upon their co-administration (Zhou et al., 2003; Bent, 2008; Zhang et al., 2015). Moreover, individuals using herbal remedies consume significantly more prescription drugs compared to nonusers, thus increasing the likelihood of herb-drug interactions (Klepser et al., 2000). Therefore, a good understanding of the underlying mechanisms of herb-drug interactions is essential for assessing and minimizing clinical risks.

## 5. Conclusions

*C. sativus* is increasingly used for its medicinal properties and has gained the interest of researchers and the public for its multiple health benefits. One important implication of the growing medicinal use of *C. sativus* is the possible interactions between its phytochemical content and the activity of enzymes involved in the biotransformation of clinically administered drugs. The present study is the first to address the effects of consumption of *C. sativus* infusion prepared from saffron of Kozani, Greece, on the *in vivo* activity of xenobiotic metabolizing enzymes in humans using caffeine as a metabolic probe. Our findings suggest that consumption of *C. sativus* for six days reduces significantly CYP1A2 activity in healthy male volunteers, but does not exert any significant effect on CYP2A6, XO and NAT2 enznyme activities. Subsequently, *C. sativus* consumption is unlikely to participate in herb-drug interactions involving medications predominantly metabolized by the enzymes studied, with the exception of potential interactions with xenobiotics metabolized by CYP1A2 in males.

## Conflicts of interest

The authors declare that they have no conflict of interests.

## Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgements

This work was funded by the Research Committee of the University of Thessaly (Code No. 4822). The funding source had no involvement in the collection, analysis and interpretation of data, in the writing of the report, and in the decision to submit the article for publication. The authors wish to thank all volunteers who participated in the study. They are also grateful to M. Daviti and N. Govaris for their excellent assistance in the experimentation.

## Transparency document

Transparency document related to this article can be found online at https://doi.org/10.1016/j.fct.2019.05.013.

## References

Alavizadeh, S.H., Hosseinzadeh, H., 2014. Bioactivity assessment and toxicity of crocin: a comprehensive review. Food Chem. Toxicol. 64, 65–80.
Altinoz, E., Oner, Z., Elbe, H., Cigremis, Y., Turkoz, Y., 2015. Protective effects of saffron (its active constituent, crocin) on nephropathy in streptozotocin-induced diabetic rats. Hum. Exp. Toxicol. 34, 127–134.
Asai, A., Nakano, T., Takahashi, M., Nagao, A., 2005. Orally administered crocetin and crocins are absorbed into blood plasma as crocetin and its glucuronide conjugates in mice. J. Agric. Food Chem. 53, 7302–7306.
Asprodini, E., Tsiokou, V., Begas, E., Kilindris, T., Kouvaras, E., Samara, M., Messinis, I., 2019. Alterations in xenobiotic-metabolizing enzyme activities across menstrual cycle in healthy volunteers. J. Pharmacol. Exp. Ther. 368, 262–271.
Asprodini, E.K., Zifa, E., Papageorgiou, I., Benakis, A., 1998. Determination of N-acetylation phenotyping in a Greek population using caffeine as a metabolic probe. Eur. J. Drug Metab. Pharmacokinet. 23, 501–506.
Assimopoulou, A.N., Sinakos, Z., Papageorgiou, V.P., 2005. Radical scavenging activity of Crocus sativus L. extract and its bioactive constituents. Phytother Res. 19, 997–1000.
Awortwe, C., Bruckmueller, H., Cascorbi, I., 2019. Interaction of herbal products with prescribed medications: a systematic review and meta-analysis. Pharmacol. Res. 141, 397–408.
Bandaranayake, W.M., 2006. Quality control, screening, toxicity, and regulation of herbal drugs. In: Modern Phytomedicine Pp 25–57. John Wiley & Sons, Ltd.
Battelli, M.G., Bolognesi, A., Polito, L., 2014. Pathophysiology of circulating xanthine oxidoreductase: new emerging roles for a multi-tasking enzyme. Biochim. Biophys. Acta 1502–1517 1842.
Begas, E., Kilindris, T., Kouvaras, E., Tsioutsioumi, A., Kouretas, D., Asprodini, E.K., 2018. Dietary effects of Sideritis scardica “mountain tea” on human in vivo activities of xenobiotic metabolizing enzymes in healthy subjects. Food Chem. Toxicol. 122, 38–48.
Begas, E., Kouvaras, E., Tsakalof, A., Papakosta, S., Asprodini, E.K., 2007. In vivo evaluation of CYP1A2, CYP2A6, NAT-2 and xanthine oxidase activities in a Greek population sample by the RP-HPLC monitoring of caffeine metabolic ratios. Biomed. Chromatogr. 21, 190–200.
Begas, E., Kouvaras, E., Tsakalof, A.K., Bounitsi, M., Asprodini, E.K., 2015. Development and validation of a reversed-phase HPLC method for CYP1A2 phenotyping by use of a caffeine metabolite ratio in saliva. Biomed Chromatogr 29, 1657–1663.
Begas, E., Tsioutsiouliti, A., Kouvaras, E., Haroutounian, S.A., Kasiotis, K.M., Kouretas, D., Asprodini, E., 2017. Effects of peppermint tea consumption on the activities of CYP1A2, CYP2A6, Xanthine Oxidase, N-acetyltranferase-2 and UDP-glucuronosyltransferases-1A1/1A6 in healthy volunteers. Food Chem. Toxicol. 100, 80–89.
Bent, S., 2008. Herbal medicine in the United States: review of efficacy, safety, and

regulation: grand rounds at University of California, San Francisco Medical Center. J. Gen. Intern. Med. 23, 854–859.
Bigos, K.L., Pollock, B.G., Coley, K.C., Miller, D.D., Marder, S.R., Aravagiri, M., Kirshner, M.A., Schneider, L.S., Bies, R.R., 2008. Sex, race, and smoking impact olanzapine exposure. J. Clin. Pharmacol. 48, 157–165.
Bisti, S., Maccarone, R., Falsini, B., 2014. Saffron and retina: neuroprotection and pharmacokinetics. Vis. Neurosci. 31, 355–361.
Bock, K.W., Schrenk, D., Forster, A., Griese, E.U., Mörike, K., Brockmeier, D., Eichelbaum, M., 1994. The influence of environmental and genetic factors on CYP2D6, CYP1A2 and UDP-glucuronosyltransferases in man using sparteine, caffeine, and paracetamol as probes. Pharmacogenetics 4, 209–218.
Bode, A.M., Dong, Z., 2009. Epigallocatechin 3-gallate and green tea catechins: united they work, divided they fail. Cancer Prev. Res. 2, 514–517.
Bodeker, G., Ong, C.-K., Grundy, C., Burford, G., Shein, K., Medicine WHOP on T, WHO Centre for Health Development, Kobe, J., 2005. WHO Global Atlas of Traditional, Complementary and Alternative Medicine. Kobe, Japan : WHO Centre for Health Development, Kobe, Japan.
Boukouvala, S., Fakis, G., 2005. Arylamine N-acetyltransferases: what we learn from genes and genomes. Drug Metab. Rev. 37, 511–564.
Campbell, M.E., Spielberg, S.P., Kalow, W., 1987. A urinary metabolite ratio that reflects systemic caffeine clearance. Clin Pharmacol Ther 42, 157–165.
Chen, Y., Liu, W.-H., Chen, B.-L., Fan, L., Han, Y., Wang, G., Hu, D.-L., Tan, Z.-R., Zhou, G., Cao, S., Zhou, H.-H., 2010. Plant polyphenol curcumin significantly affects CYP1A2 and CYP2A6 activity in healthy, male Chinese volunteers. Ann. Pharmacother. 44, 1038–1045.
Chen, Y., Xiao, C.-Q., He, Y.-J., Chen, B.-L., Wang, G., Zhou, G., Zhang, W., Tan, Z.-R., Cao, S., Wang, L.-P., Zhou, H.-H., 2011. Genistein alters caffeine exposure in healthy female volunteers. Eur. J. Clin. Pharmacol. 67, 347–353.
Chen, Y., Xiao, P., Ou-Yang, D.-S., Fan, L., Guo, D., Wang, Y.-N., Han, Y., Tu, J.-H., Zhou, G., Huang, Y.-F., Zhou, H.-H., 2009. Simultaneous action of the flavonoid quercetin on cytochrome P450 (CYP) 1A2, CYP2A6, N-acetyltransferase and xanthine oxidase activity in healthy volunteers. Clin. Exp. Pharmacol. Physiol. 36, 828–833.
Chiney, M.S., Schwarzenberg, S.J., Johnson, L.A., 2011. Altered xanthine oxidase and N-acetyltransferase activity in obese children. Br. J. Clin. Pharmacol. 72, 109–115.
Christodoulou, E., Kadoglou, N.P.E., Kostomitsopoulos, N., Valsami, G., 2015. Saffron: a natural product with potential pharmaceutical applications. J. Pharm. Pharmacol. 67, 1634–1649.
Chryssanthi, D.G., Lamari, F.N., Georgakopoulos, C.D., Cordopatis, P., 2011. A new validated SPE-HPLC method for monitoring crocetin in human plasma–application after saffron tea consumption. J. Pharm. Biomed. Anal. 55, 563–568.
Di, Y.M., Chow, V.D.-W., Yang, L.-P., Zhou, S.-F., 2009. Structure, function, regulation and polymorphism of human cytochrome P450 2A6. Curr. Drug Metabol. 10, 754–780.
Dovrtělová, G., Nosková, K., Juřica, J., Turjap, M., Zendulka, O., 2015. Can bioactive compounds of Crocus sativus L. influence the metabolic activity of selected CYP enzymes in the rat? Physiol. Res. 64 (Suppl. 4), S453–S458.
Dupret, J.-M., Rodrigues-Lima, F., 2005. Structure and regulation of the drug-metabolizing enzymes arylamine N-acetyltransferases. Curr. Med. Chem. 12, 311–318.
Ekor, M., 2014. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. Front. Pharmacol. 4, 177.
Ekstrand, B., Rasmussen, M.K., Woll, F., Zlabek, V., Zamaratskaia, G., 2015. In vitro gender-dependent inhibition of porcine cytochrome p450 activity by selected flavonoids and phenolic acids. BioMed Res. Int. 2015, 387918.
Evans, D.A., 1989. N-acetyltransferase. Pharmacol. Ther. 42, 157–234.
Fuhr, U., Rost, K.L., 1994. Simple and reliable CYP1A2 phenotyping by the paraxanthine/caffeine ratio in plasma and in saliva. Pharmacogenetics 4, 109–116.
García-Rodríguez, M.V., López-Córcoles, H., Alonso, G.L., Pappas, C.S., Polissiou, M.G., Tarantilis, P.A., 2017. Comparative evaluation of an ISO 3632 method and an HPLC-DAD method for safranal quantity determination in saffron. Food Chem. 221, 838–843.
Ghasemi, T., Abnous, K., Vahdati, F., Mehri, S., Razavi, B.M., Hosseinzadeh, H., 2015. Antidepressant effect of crocus sativus aqueous extract and its effect on CREB, BDNF, and VGF transcript and protein levels in rat Hippocampus. Drug Res. 65, 337–343.
Gu, L., Gonzalez, F.J., Kalow, W., Tang, B.K., 1992. Biotransformation of caffeine, paraxanthine, theobromine and theophylline by cDNA-expressed human CYP1A2 and CYP2E1. Pharmacogenetics 2, 73–77.
Gurley, B.J., Gardner, S.F., Hubbard, M.A., Williams, D.K., Gentry, W.B., Cui, Y., Ang, C.Y.W., 2005. Clinical assessment of effects of botanical supplementation on cytochrome P450 phenotypes in the elderly: St John's wort, garlic oil, Panax ginseng and Ginkgo biloba. Drugs Aging 22, 525–539.
Gurley, B.J., Gardner, S.F., Hubbard, M.A., Williams, D.K., Gentry, W.B., Cui, Y., Ang, C.Y.W., 2002. Cytochrome P450 phenotypic ratios for predicting herb-drug interactions in humans. Clin. Pharmacol. Ther. 72, 276–287.
Hakooz, N.M.K., 2009. Caffeine metabolic ratios for the in vivo evaluation of CYP1A2, N-acetyltransferase 2, xanthine oxidase and CYP2A6 enzymatic activities. Curr. Drug Metabol. 10, 329–338.
Hammerness, P., Basch, E., Ulbricht, C., Barrette, E.-P., Foppa, I., Basch, S., Bent, S., Boon, H., Ernst, E., Natural Standard Research Collaboration, 2003. St John's wort: a systematic review of adverse effects and drug interactions for the consultation psychiatrist. Psychosomatics 44, 271–282.
Harrison, R., 2002. Structure and function of xanthine oxidoreductase: where are we now? Free Radic. Biol. Med. 33, 774–797.
Hassan, M.H., Bahashawan, S.A., Abdelghany, T.M., Abd-Allah, G.M., Ghobara, M.M., 2015. Crocin abrogates carbon tetrachloride-induced renal toxicity in rats via modulation of metabolizing enzymes and diminution of oxidative stress, apoptosis, and inflammatory cytokines. J. Biochem. Mol. Toxicol. 29, 330–339.
Hatziagapiou, K., Lambrou, G.I., 2018. The protective role of crocus sativus L. (Saffron) against ischemia- reperfusion injury, hyperlipidemia and atherosclerosis: nature opposing cardiovascular diseases. Curr. Cardiol. Rev. 14, 272–289.
Hausenblas, H.A., Heekin, K., Mutchie, H.L., Anton, S., 2015. A systematic review of randomized controlled trials examining the effectiveness of saffron (Crocus sativus L.) on psychological and behavioral outcomes. J Integr Med 13, 231–240.
Hein, D.W., Fakis, G., Boukouvala, S., 2018. Functional expression of human arylamine N-acetyltransferase NAT1*10 and NAT1*11 alleles: a mini review. Pharmacogenetics Genom. 28, 238–244.
Hoshyar, R., Mollaei, H., 2017. A comprehensive review on anticancer mechanisms of the main carotenoid of saffron, crocin. J. Pharm. Pharmacol. 69, 1419–1427.
Islam, M.M., Iqbal, U., Walther, B.A., Nguyen, P.-A., Li, Y.-C.J., Dubey, N.K., Poly, T.N., Masud, J.H.B., Atique, S., Syed-Abdul, S., 2017. Gender-based personalized pharmacotherapy: a systematic review. Arch. Gynecol. Obstet. 295, 1305–1317.
Jewell, C., O'Brien, N.M., 1999. Effect of dietary supplementation with carotenoids on xenobiotic metabolizing enzymes in the liver, lung, kidney and small intestine of the rat. Br. J. Nutr. 81, 235–242.
Jiang, Z., Dragin, N., Jorge-Nebert, L.F., Martin, M.V., Guengerich, F.P., Aklillu, E., Ingelman-Sundberg, M., Hammons, G.J., Lyn-Cook, B.D., Kadlubar, F.F., Saldana, S.N., Sorter, M., Vinks, A.A., Nassr, N., von Richter, O., Jin, L., Nebert, D.W., 2006. Search for an association between the human CYP1A2 genotype and CYP1A2 metabolic phenotype. Pharmacogenetics Genom. 16, 359–367.
Kalow, W., Tang, B.K., 1991. Use of caffeine metabolite ratios to explore CYP1A2 and xanthine oxidase activities. Clin Pharmacol Ther 50, 508–519.
Kamalipour, M., Akhondzadeh, S., 2011. Cardiovascular effects of saffron: an evidence-based review. J Tehran Heart Cent 6, 59–61.
Kamimori, G.H., Joubert, A., Otterstetter, R., Santaromana, M., Eddington, N.D., 1999. The effect of the menstrual cycle on the pharmacokinetics of caffeine in normal, healthy eumenorrheic females. Eur. J. Clin. Pharmacol. 55, 445–449.
Karkoula, E., Angelis, A., Koulakiotis, N.-S., Gikas, E., Halabalaki, M., Tsarbopoulos, A., Skaltsounis, A.-L., 2018. Rapid isolation and characterization of crocins, picrocrocin, and crocetin from saffron using centrifugal partition chromatography and LC-MS. J. Sep. Sci. 41, 4105–4114.
Kashuba, A.D.M., Bertino, J.S., Kearns, G.L., Leeder, J.S., James, A.W., Gotschall, R., Nafziger, A.N., 1998. Quantitation of three-month intraindividual variability and influence of sex and menstrual cycle phase on CYP1A2, N-acetyltransferase-2, and xanthine oxidase activity determined with caffeine phenotyping. Clin. Pharmacol. Therapeut. 63, 540–551.
Kelly, D.L., Conley, R.R., Tamminga, C.A., 1999. Differential olanzapine plasma concentrations by sex in a fixed-dose study. Schizophr. Res. 40, 101–104.
Kelly, D.L., Richardson, C.M., Yu, Y., Conley, R.R., 2006. Plasma concentrations of high-dose olanzapine in a double-blind crossover study. Hum. Psychopharmacol. 21, 393–398.
Khorasanchi, Z., Shafiee, M., Kermanshahi, F., Khazaei, M., Ryzhikov, M., Parizadeh, M.R., Kermanshahi, B., Ferns, G.A., Avan, A., Hassanian, S.M., 2018. Crocus sativus a natural food coloring and flavoring has potent anti-tumor properties. Phytomedicine 43, 21–27.
Klepser, T.B., Doucette, W.R., Horton, M.R., Buys, L.M., Ernst, M.E., Ford, J.K., Hoehns, J.D., Kautzman, H.A., Logemann, C.D., Swegle, J.M., Ritho, M., Klepser, M.E., 2000. Assessment of patients' perceptions and beliefs regarding herbal therapies. Pharmacotherapy 20, 83–87.
Klisic, A., Kocic, G., Kavaric, N., Jovanovic, M., Stanisic, V., Ninic, A., 2018. Body mass index is independently associated with xanthine oxidase activity in overweight/obese population. Eat. Weight Disord. https://doi.org/10.1007/s40519-018-0490-5.
Koonrungsesomboon, N., Khatsri, R., Wongchompoo, P., Teekachunhatean, S., 2018. The impact of genetic polymorphisms on CYP1A2 activity in humans: a systematic review and meta-analysis. Pharmacogenomics J. 18, 760–768.
Kot, M., Daniel, W.A., 2008. The relative contribution of human cytochrome P450 isoforms to the four caffeine oxidation pathways: an in vitro comparative study with cDNA-expressed P450s including CYP2C isoforms. Biochem. Pharmacol. 76, 543–551.
Krenitsky, T.A., Neil, S.M., Elion, G.B., Hitchings, G.H., 1972. A comparison of the specificities of xanthine oxidase and aldehyde oxidase. Arch. Biochem. Biophys. 150, 585–599.
Kukongviriyapan, V., Phromsopha, N., Tassaneeyakul, W., Kukongviriyapan, U., Sripa, B., Hahnvajanawong, V., Bhudhisawasdi, V., 2006. Inhibitory effects of polyphenolic compounds on human arylamine N-acetyltransferase 1 and 2. Xenobiotica 36, 15–28.
Kyriakoudi, A., O'Callaghan, Y.C., Galvin, K., Tsimidou, M.Z., O'Brien, N.M., 2015. Cellular transport and bioactivity of a major saffron apocarotenoid, picrocrocin (4-(β-D-glucopyranosyloxy)-2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde). J. Agric. Food Chem. 63, 8662–8668.
Lashay, A., Sadough, G., Ashrafi, E., Lashay, M., Movassat, M., Akhondzadeh, S., 2016. Short-term outcomes of saffron supplementation in patients with age-related macular degeneration: a double-blind, placebo-controlled, randomized trial. Med. Hypothesis, Discov. Innovation (MEHDI) Ophthalmol. 5, 32–38.
Le Marchand, L., Franke, A.A., Custer, L., Wilkens, L.R., Cooney, R.V., 1997. Lifestyle and nutritional correlates of cytochrome CYP1A2 activity: inverse associations with plasma lutein and alpha-tocopherol. Pharmacogenetics 7, 11–19.
Lelo, A., Miners, J.O., Robson, R.A., Birkett, D.J., 1986. Quantitative assessment of caffeine partial clearances in man. Br. J. Clin. Pharmacol. 22, 183–186.
Leone, S., Recinella, L., Chiavaroli, A., Orlando, G., Ferrante, C., Leporini, L., Brunetti, L., Menghini, L., 2018. Phytotherapic use of the Crocus sativus L. (Saffron) and its potential applications: a brief overview. Phytother Res. 32, 2364–2375.
Li, W., 2002. Botanical drugs: a future for herbal medicines. J. Contemp. Health Law Policy 19, 117–149.
Linardaki, Z.I., Orkoula, M.G., Kokkosis, A.G., Lamari, F.N., Margarity, M., 2013.

Investigation of the neuroprotective action of saffron (Crocus sativus L.) in aluminum-exposed adult mice through behavioral and neurobiochemical assessment. Food Chem. Toxicol. 52, 163–170.
Liu, C.-C., Huang, C.-C., Lin, W.-T., Hsieh, C.-C., Huang, S.-Y., Lin, S.-J., Yang, S.-C., 2005. Lycopene supplementation attenuated xanthine oxidase and myeloperoxidase activities in skeletal muscle tissues of rats after exhaustive exercise. Br. J. Nutr. 94, 595–601.
Liu, T.-T., Liang, N.-S., Li, Y., Yang, F., Lu, Y., Meng, Z.-Q., Zhang, L.-S., 2003. Effects of long-term tea polyphenols consumption on hepatic microsomal drug-metabolizing enzymes and liver function in Wistar rats. World J. Gastroenterol. 9, 2742–2744.
López-Flores, L.A., Pérez-Rubio, G., Falfán-Valencia, R., 2017. Distribution of polymorphic variants of CYP2A6 and their involvement in nicotine addiction. EXCLI J 16, 174–196.
Lopresti, A.L., Drummond, P.D., 2017. Efficacy of curcumin, and a saffron/curcumin combination for the treatment of major depression: a randomised, double-blind, placebo-controlled study. J. Affect. Disord. 207, 188–196.
Lopresti, A.L., Drummond, P.D., 2014. Saffron (Crocus sativus) for depression: a systematic review of clinical studies and examination of underlying antidepressant mechanisms of action. Hum. Psychopharmacol. 29, 517–527.
Louisa, M., Suyatna, F.D., Setiawati, A., Jusman, S.W.A., 2009. The effect of lycopene on the total cytochrome P450, CYP1A2 and CYP2E1. Medical Journal of Indonesia 18, 233–238.
Marazziti, D., Baroni, S., Picchetti, M., Piccinni, A., Carlini, M., Vatteroni, E., Falaschi, V., Lombardi, A., Dell'Osso, L., 2013. Pharmacokinetics and pharmacodinamics of psychotropic drugs: effect of sex. CNS Spectr. 18, 118–127.
Marín, L., Miguélez, E.M., Villar, C.J., Lombó, F., 2015. Bioavailability of dietary polyphenols and gut microbiota metabolism: antimicrobial properties. BioMed Res. Int. 2015, 905215.
Meibohm, B., Beierle, I., Derendorf, H., 2002. How important are gender differences in pharmacokinetics? Clin. Pharmacokinet. 41, 329–342.
Melnyk, J.P., Wang, S., Marcone, M.F., 2010. Chemical and biological properties of the world's most expensive spice: Saffron. Food Res. Int. 43, 1981–1989.
Milajerdi, A., Jazayeri, S., Hashemzadeh, N., Shirzadi, E., Derakhshan, Z., Djazayeri, A., Akhondzadeh, S., 2018. The effect of saffron (Crocus sativus L.) hydroalcoholic extract on metabolic control in type 2 diabetes mellitus: a triple-blinded randomized clinical trial. J. Res. Med. Sci. 23, 16.
Moon, Y.J., Wang, X., Morris, M.E., 2006. Dietary flavonoids: effects on xenobiotic and carcinogen metabolism. Toxicol. Vitro 20, 187–210.
Moore, L.B., Goodwin, B., Jones, S.A., Wisely, G.B., Serabjit-Singh, C.J., Willson, T.M., Collins, J.L., Kliewer, S.A., 2000. St. John's wort induces hepatic drug metabolism through activation of the pregnane X receptor. Proc. Natl. Acad. Sci. U. S. A. 97, 7500–7502.
Mousavi, S.H., Tavakkol-Afshari, J., Brook, A., Jafari-Anarkooli, I., 2009. Role of caspases and Bax protein in saffron-induced apoptosis in MCF-7 cells. Food Chem. Toxicol. 47, 1909–1913.
NAGAO, A., SEKI, M., KOBAYASHI, H., 1999. Inhibition of xanthine oxidase by flavonoids. Biosci. Biotechnol. Biochem. 63, 1787–1790.
Nagata, K., Ishitobi, K., Yamamoto, Y., Ikeda, T., Hori, S., Matsumoto, Y., Sasaki, T., 1997. Increased theophylline metabolism in the menstrual phase of healthy women. J. Allergy Clin. Immunol. 100, 39–43.
Nehlig, A., 2018. Interindividual differences in caffeine metabolism and factors driving caffeine consumption. Pharmacol. Rev. 70, 384–411.
Ou-Yang, D.S., Huang, S.L., Wang, W., Xie, H.G., Xu, Z.H., Shu, Y., Zhou, H.H., 2000. Phenotypic polymorphism and gender-related differences of CYP1A2 activity in a Chinese population. Br. J. Clin. Pharmacol. 49, 145–151.
Papandreou, M.A., Kanakis, C.D., Polissiou, M.G., Efthimiopoulos, S., Cordopatis, P., Margarity, M., Lamari, F.N., 2006. Inhibitory activity on amyloid-beta aggregation and antioxidant properties of Crocus sativus stigmas extract and its crocin constituents. J. Agric. Food Chem. 54, 8762–8768.
Papandreou, M.A., Tsachaki, M., Efthimiopoulos, S., Cordopatis, P., Lamari, F.N., Margarity, M., 2011. Memory enhancing effects of saffron in aged mice are correlated with antioxidant protection. Behav. Brain Res. 219, 197–204.
Pelkonen, O., Turpeinen, M., Hakkola, J., Honkakoski, P., Hukkanen, J., Raunio, H., 2008. Inhibition and induction of human cytochrome P450 enzymes: current status. Arch. Toxicol. 82, 667–715.
Pitsikas, N., 2015. The effect of crocus sativus L. And its constituents on memory: basic studies and clinical applications. Evid Based Complement Alternat Med 2015, 926284.
Pitsikas, N., Sakellaridis, N., 2006. Crocus sativus L. extracts antagonize memory impairments in different behavioural tasks in the rat. Behav. Brain Res. 173, 112–115.
Pitsikas, N., Zisopoulou, S., Tarantilis, P.A., Kanakis, C.D., Polissiou, M.G., Sakellaridis, N., 2007. Effects of the active constituents of Crocus sativus L., crocins on recognition and spatial rats' memory. Behav. Brain Res. 183, 141–146.
Pourmasoumi, M., Hadi, A., Najafgholizadeh, A., Kafeshani, M., Sahebkar, A., 2019. Clinical evidence on the effects of saffron (Crocus sativus L.) on cardiovascular risk factors: a systematic review meta-analysis. Pharmacol. Res. 139, 348–359.
Pritsos, C.A., 2000. Cellular distribution, metabolism and regulation of the xanthine oxidoreductase enzyme system. Chem. Biol. Interact. 129, 195–208.
Ravindran, A.V., Balneaves, L.G., Faulkner, G., Ortiz, A., McIntosh, D., Morehouse, R.L., Ravindran, L., Yatham, L.N., Kennedy, S.H., Lam, R.W., MacQueen, G.M., Milev, R.V., Parikh, S.V., CANMAT Depression Work Group, 2016. Canadian Network for Mood and anxiety treatments (CANMAT) 2016 clinical guidelines for the management of adults with major depressive disorder: section 5. Complementary and alternative medicine treatments. Can. J. Psychiatr. 61, 576–587.
Relling, M.V., Lin, J.S., Ayers, G.D., Evans, W.E., 1992. Racial and gender differences in N-acetyltransferase, xanthine oxidase, and CYP1A2 activities. Clin. Pharmacol. Ther. 52, 643–658.
Rendic, S., Di Carlo, F.J., 1997. Human cytochrome P450 enzymes: a status report summarizing their reactions, substrates, inducers, and inhibitors. Drug Metab. Rev. 29, 413–580.
Rostami-Hodjegan, A., Nurminen, S., Jackson, P.R., Tucker, G.T., 1996. Caffeine urinary metabolite ratios as markers of enzyme activity: a theoretical assessment. Pharmacogenetics 6, 121–149.
Sánchez, A.M., Carmona, M., Zalacain, A., Carot, J.M., Jabaloyes, J.M., Alonso, G.L., 2008. Rapid determination of crocetin esters and picrocrocin from saffron spice (Crocus sativus L.) using UV-visible spectrophotometry for quality control. J. Agric. Food Chem. 56, 3167–3175.
Saruwatari, J., Takaishi, C., Yoshida, K., Takashima, A., Fujimura, Y., Umemoto, Y., Abe, T., Kitamado, M., Shimomasuda, M., Muramoto, Y., Nakagawa, K., 2012. A herbal-drug interaction study of keishi-bukuryo-gan, a traditional herbal preparation used for menopausal symptoms, in healthy female volunteers. J. Pharm. Pharmacol. 64, 670–676.
Schliep, K.C., Schisterman, E.F., Wactawski-Wende, J., Perkins, N.J., Radin, R.G., Zarek, S.M., Mitchell, E.M., Sjaarda, L.A., Mumford, S.L., 2016. Serum caffeine and paraxanthine concentrations and menstrual cycle function: correlations with beverage intakes and associations with race, reproductive hormones, and anovulation in the BioCycle Study. Am. J. Clin. Nutr. 104, 155–163.
Schmidt, M., Betti, G., Hensel, A., 2007. Saffron in phytotherapy: pharmacology and clinical uses. Wien Med. Wochenschr. 157, 315–319.
Shafiee, M., Arekhi, S., Omranzadeh, A., Sahebkar, A., 2018. Saffron in the treatment of depression, anxiety and other mental disorders: current evidence and potential mechanisms of action. J. Affect. Disord. 227, 330–337.
Shimada, H., Eto, M., Ohtaguro, M., Ohtsubo, M., Mizukami, Y., Ide, T., Imamura, Y., 2010. Differential mechanisms for the inhibition of human cytochrome P450 1A2 by apigenin and genistein. J. Biochem. Mol. Toxicol. 24, 230–234.
Shimada, T., Yamazaki, H., Mimura, M., Inui, Y., Guengerich, F.P., 1994. Interindividual variations in human liver cytochrome P-450 enzymes involved in the oxidation of drugs, carcinogens and toxic chemicals: studies with liver microsomes of 30 Japanese and 30 Caucasians. J. Pharmacol. Exp. Ther. 270, 414–423.
Sim, E., Fakis, G., Laurieri, N., Boukouvala, S., 2012. Arylamine N-acetyltransferases–from drug metabolism and pharmacogenetics to identification of novel targets for pharmacological intervention. Adv. Pharmacol. 63, 169–205.
Sobolev, A.P., Carradori, S., Capitani, D., Vista, S., Trella, A., Marini, F., Mannina, L., 2014. Saffron samples of different Origin: an NMR study of microwave-assisted extracts. Foods 3, 403–419.
Tantcheva-Poór, I., Zaigler, M., Rietbrock, S., Fuhr, U., 1999. Estimation of cytochrome P-450 CYP1A2 activity in 863 healthy Caucasians using a saliva-based caffeine test. Pharmacogenetics 9, 131–144.
Tarantilis, P.A., Tsoupras, G., Polissiou, M., 1995. Determination of saffron (Crocus sativus L.) components in crude plant extract using high-performance liquid chromatography-UV-visible photodiode-array detection-mass spectrometry. J. Chromatogr. A 699, 107–118.
Tavakkol-Afshari, J., Brook, A., Mousavi, S.H., 2008. Study of cytotoxic and apoptogenic properties of saffron extract in human cancer cell lines. Food Chem. Toxicol. 46, 3443–3447.
Tiong, K.H., Yiap, B.C., Tan, E.L., Ismail, R., Ong, C.E., 2010. In vitro modulation of naturally occurring flavonoids on cytochrome P450 2A6 (CYP2A6) activity. Xenobiotica 40, 458–466.
Tomankova, V., Liskova, B., Skalova, L., Bartikova, H., Bousova, I., Jourova, L., Anzenbacher, P., Ulrichova, J., Anzenbacherova, E., 2015. Altered cytochrome P450 activities and expression levels in the liver and intestines of the monosodium glutamate-induced mouse model of human obesity. Life Sci. 133, 15–20.
Tóth, B., Hegyi, P., Lantos, T., Szakács, Z., Kerémi, B., Varga, G., Tenk, J., Pétervári, E., Balaskó, M., Rumbus, Z., Rakonczay, Z., Bálint, E.R., Kiss, T., Csupor, D., 2019. The efficacy of saffron in the treatment of mild to moderate depression: a meta-analysis. Planta Med. 85, 24–31.
Tsolaki, M., Karathanasi, E., Lazarou, I., Dovas, K., Verykouki, E., Karacostas, A., Georgiadis, K., Tsolaki, A., Adam, K., Kompatsiaris, I., Sinakos, Z., 2016. Efficacy and safety of crocus sativus L. In patients with mild cognitive impairment: one year single-blind randomized, with parallel groups, clinical trial. J. Alzheimer's Dis. 54, 129–133.
Uchida, S., Yamada, H., Li, X.D., Maruyama, S., Ohmori, Y., Oki, T., Watanabe, H., Umegaki, K., Ohashi, K., Yamada, S., 2006. Effects of Ginkgo biloba extract on pharmacokinetics and pharmacodynamics of tolbutamide and midazolam in healthy volunteers. J. Clin. Pharmacol. 46, 1290–1298.
Wang, H., Leung, L.K., 2010. The carotenoid lycopene differentially regulates phase I and II enzymes in dimethylbenz[a]anthracene-induced MCF-7 cells. Nutrition 26, 1181–1187.
Wang, Y., Han, T., Zhu, Y., Zheng, C.-J., Ming, Q.-L., Rahman, K., Qin, L.-P., 2010. Antidepressant properties of bioactive fractions from the extract of Crocus sativus L. J. Nat. Med. 64, 24–30.
Wang, Z., Gorski, J.C., Hamman, M.A., Huang, S.M., Lesko, L.J., Hall, S.D., 2001. The effects of St John's wort (Hypericum perforatum) on human cytochrome P450 activity. Clin. Pharmacol. Ther. 70, 317–326.
Wanwimolruk, S., Prachayasittikul, V., 2014. Cytochrome P450 enzyme mediated herbal drug interactions (Part 1). EXCLI J 13, 347–391.
Weisburger, J.H., Dolan, L., Pittman, B., 1998. Inhibition of PhIP mutagenicity by caffeine, lycopene, daidzein, and genistein. Mutat. Res. 416, 125–128.
Wenk, M., Todesco, L., Krähenbühl, S., 2004. Effect of St John's wort on the activities of CYP1A2, CYP3A4, CYP2D6, N-acetyltransferase 2, and xanthine oxidase in healthy males and females. Br. J. Clin. Pharmacol. 57, 495–499.
Williamson, E.M., 2001. Synergy and other interactions in phytomedicines.

Phytomedicine 8, 401–409.
Wong, P., Villeneuve, G., Tessier, V., Banerjee, K., Nedev, H., Jean-Claude, B.J., Leyland-Jones, B., 2002. Stability of 5-acetamido-6-formylamino-3-methyluracil in buffers and urine. J Pharm Biomed Anal 28, 693–700.
Wu, J.-C., Lai, C.-S., Tsai, M.-L., Ho, C.-T., Wang, Y.-J., Pan, M.-H., 2017. Chemopreventive effect of natural dietary compounds on xenobiotic-induced toxicity. J. Food Drug Anal. 25, 176–186.
Xi, L., Qian, Z., Du, P., Fu, J., 2007. Pharmacokinetic properties of crocin (crocetin digentiobiose ester) following oral administration in rats. Phytomedicine 14, 633–636.
Yang, X., Chen, X., Fu, Y., Luo, Q., Du, L., Qiu, H., Qiu, T., Zhang, L., Meng, H., 2018. Comparative efficacy and safety of Crocus sativus L. for treating mild to moderate major depressive disorder in adults: a meta-analysis of randomized controlled trials. Neuropsychiatric Dis. Treat. 14, 1297–1305.
Yaribeygi, H., Zare, V., Butler, A.E., Barreto, G.E., Sahebkar, A., 2019. Antidiabetic potential of saffron and its active constituents. J. Cell. Physiol. 234, 8610–8617.
Yi, S., Cho, J.-Y., Lim, K.S., Kim, K., Kim, J., Kim, B.-H., Hong, J., Jang, I.-J., Shin, S.-G., Yu, K.-S., 2009. Effects of Angelicae tenuissima radix, Angelicae dahuricae radix and Scutellariae radix extracts on cytochrome P450 activities in healthy volunteers. Basic Clin. Pharmacol. Toxicol. 105, 249–256.
Zabela, V., Sampath, C., Oufir, M., Moradi-Afrapoli, F., Butterweck, V., Hamburger, M., 2016. Pharmacokinetics of dietary kaempferol and its metabolite 4-hydroxyphenylacetic acid in rats. Fitoterapia 115, 189–197.
Zhang, J., Onakpoya, I.J., Posadzki, P., Eddouks, M., 2015. The safety of herbal medicine: from prejudice to evidence. Evid Based Complement Alternat Med 316706 2015.
Zhang, Y., Fei, F., Zhen, L., Zhu, X., Wang, J., Li, S., Geng, J., Sun, R., Yu, X., Chen, T., Feng, S., Wang, P., Yang, N., Zhu, Y., Huang, J., Zhao, Y., Aa, J., Wang, G., 2017. Sensitive analysis and simultaneous assessment of pharmacokinetic properties of crocin and crocetin after oral administration in rats. J Chromatogr B Analyt Technol Biomed Life Sci 1044–1045, 1–7.
Zhang, Z., Wang, C.-Z., Wen, X.-D., Shoyama, Y., Yuan, C.-S., 2013. Role of saffron and its constituents on cancer chemoprevention. Pharm. Biol. 51, 920–924.
Zhou, S., Gao, Y., Jiang, W., Huang, M., Xu, A., Paxton, J.W., 2003. Interactions of herbs with cytochrome P450. Drug Metab. Rev. 35, 35–98.
Zhou, S.-F., Wang, B., Yang, L.-P., Liu, J.-P., 2010. Structure, function, regulation and polymorphism and the clinical significance of human cytochrome P450 1A2. Drug Metab. Rev. 42, 268–354.