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Research Paper

# *Laurencia caspica* Extract Strongly Induces *CYP2D6* Transcription and Stabilizes Diazinon-induced Transcriptional Fluctuations in AGS Cells

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## ABSTRACT

**Background:** Cytochrome P450 (CYP) isoform 2D6, the second most abundant CYP enzyme in the body involving in the oxidative metabolism of a large number of endogenous and exogenous compounds, is highly relevant to transcriptional alteration-mediated carcinogenesis. However, the absence of a known biological inducer, in addition to its frequent transcriptional changes, has made its application challenging. The transactivating potential of *Laurencia caspica* extract for *CYP2D6* transcription was evaluated in AGS cells in an attempt to correct its diazinon-induced fluctuations.

**Materials and Methods:** The cytotoxicity of diazinon and Iethanol extract was studied in AGS cells using the MTT assay. *CYP2D6* mRNA levels were quantitatively estimated using real-time polymerase chain reaction (PCR) analysis after treatment of the cells with 0–10 µM concentrations of diazinon or 0–40 µg/mL of *L. caspica* extract, as well as after their co-treatment. The latter treatment, which involved treating cells with 0.1 µM diazinon after pretreatment with various concentrations of *L. caspica* extract, was designed to evaluate the protective efficacy of *L. caspica* extract against *CYP2D6* transcriptional changes induced by diazinon treatment. All tests were carried out three times, each in triplicate, and the results expressed as Mean±SD.

**Results:** The MTT assay revealed $IC_{50}$ values of 80 µM and 840 µg/mL for diazinon and *L. capsica* extract, respectively. Interestingly, *L. capsica* extract showed proliferative activity at the concentrations up to 70 µg/mL. The qRT-PCR results from the independent experiments involving diazinon treatment revealed irregular transcription fluctuations in *CYP2D6* transcription. However, the mean of all the data produced a bell-shaped curve, in which treatment with 0 and 10 µM diazinon caused the greatest downregulation of *CYP2D6*, whereas its transcription remained unchanged following treatment with 1 µM diazinon. Treatment with *L. caspica* extract strongly and steadily induced *CYP2D6* transcription, resulting in a 4-fold increase in mRNA levels at the highest extract concentration (40 µg/mL). Similarly, treatment of the cells with 0.1 µM diazinon after pretreatment with various concentrations of *L. caspica* extract revealed dose-dependent transcription up-regulation similar to that induced by *L. caspica* alone, particularly at higher concentrations.

**Conclusion:** Ethanol extract from *L. caspica* has antioxidant and proliferative activities at lower concentrations. It is a strong inducer of *CYP2D6* transcription and may correct diazinon-induced *CYP2D6* fluctuations. Bioactive molecules from *L. caspica* are promising candidates for the detoxification of chemotherapy drugs, particularly in patients with gastric cancer.

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## Introduction

Gastric cancer (GC) is the fifth most common and the third deadliest malignancy worldwide, with over one million new cases and approximately 770,000 cancer-related deaths estimated in 2020 [1]. The incidence rate of GC is highly variable based on region, culture, and dietary or environmental risk factors. The highest incidence rates have been reported in eastern and central Asia and North and Latin America, while regions in northern and eastern Africa have the lowest rates [2]. Similarly, variable incidence and mortality rates for the disease have been reported from different regions of Iran, with the northern parts of the country, located along the southern coastal areas of the Caspian Sea, estimated to have the highest rates [3]. Worldwide recognition of the GC risk factors and gradual socio-economic improvements in Asia have decreased the incidence and mortality rates of the disease over the past decades. However, this decrease has been less marked in populations from some developed and developing countries. This may indicate the introduction of new risk factors or the reemergence of the previously known causes of the disease, such as infection with *Helicobacter pylori*, gastritis, other dietary and environmental risk factors, and genetic polymorphisms [4]. Northern Iran, the country's main region for crop production, experiences the overuse of agricultural pesticides; as a result, as much as 3,000 tons of pesticides are applied to agricultural fields every year [5]. Residual agricultural pesticides have frequently been reported in various agricultural sources and, shockingly, in blood samples from both diseased and healthy individuals [6].

Epidemiological studies have robustly linked organophosphates (OP) exposure with the increased risk of various cancers [7]. Diazinon, one of the most widely used OP pesticides, is directly associated with the occurrence of lung cancer and has been related to other malignancies, such as gastric, prostate, and breast cancers [8-11]. Most xenobiotics, such as pesticides undergo phase I and phase II biotransformation reactions that produce hydrophilic metabolites that are directly eliminated by the kidneys [12]. A group of heme-containing enzymes known as cytochrome P450 (CYP) is substantially involved in phase I oxidative reactions [13]. Human CYPs have broad substrate specificity, and members of families 1–3—particularly CYP1A1, CYP1A2, CYP2B6, CYP2D6, CYP2C9, CYP2C19, CYP3A4, and CYP3A5—are primarily involved in the deactivation of drugs and procarcinogens and the detoxification of xenobiotic substances [14, 15]. CYPs are primarily found in hepatic cells; however, their extrahepatic activities occur in a variety of tissues, such as the gastrointestinal tract, brain, and kidneys [16].

CYP2D6, as the prototype member of the family 2, s one of the most abundant CYP enzymes in the body, along with CYP3A4. It is involved in the metabolism of about 30% of the exogenous and endogenous compounds, despite constituting only 2-4% of all hepatic CYP content [17]. CYP2D6 activity may affect cell growth and proliferation through the accidental production of the DNA-damaging compounds generated during the detoxification steps of the procarcinogens [18]. The *CYP2D6* gene is highly polymorphic, with more than 90 inter-ethnic and inter-population allelic variants. CYP2D6 activity ranges from ultrarapid metabolizers (UMs), extensive metabolizers (EMs), intermediate metabolizers (IMs) and poor metabolizers (PMs) among different ethnic groups. Although *CYP2D6* has been widely considered to be non-inducible based on human hepatocyte studies, data from in vivo assays suggest that it is inducible by endogenous and exogenous compounds [19]. In particular, studies on human liver microsomes have revealed that CYP2D6 is an important enzyme in the bioactivation of the Ops, like diazinon. Conversely, diazinon disrupts the mitochondrial membrane delivery system in rat liver and damages CYP enzymes in the human liver [20]. Moreover, in vitro and animal studies have shown that Ops, like diazinon disrupt CYP expression through promoter alkylation, which could lead to malignancies such as leukemia and non-Hodgkin's lymphoma (NHL), as well as prostate, brain, and lung cancers [21-23]. We recently reported that diazinon may involve in the incidence of GC through the epigenetic alteration of *CYP3A4* transcription [11].

Various traditional approaches, such as surgery, radiotherapy, and chemotherapy have been used to treat cancer, but the outcomes have unfortunately been very poor in most cases and associated with undesirable side effects [24]. Natural products, including alkaloids and polyphenols from plant, algae, and fungus resources offer distinct advantages for cancer chemotherapy [25-27]. It was reported that the secondary metabolites from the genus *Laurencia* of red algae have the potential to be used as anti-inflammatory and anti-cancer drugs [28-30].

Given the emerging role of pesticides in the etiology of gastric cancer, together with the widespread involvement of CYP2D6 in the metabolism of exogenous compounds and the resulting transcriptional alterations, we sought to study changes in *CYP2D6* transcription following treatment of AGS cells with the pesticide diazinon and *L. caspica* extract.

## Materials and Methods

### Cell lines and culture media

AGS cells (Pasteur Ins., Iran) were cultured in dulbecco's modified eagle's medium-high glucose (DMEM) (BioIdea, Iran) containing 15% FBS (BioIdea, Iran) and Pen/Strep antibiotics (100 U/mL penicillin and 100 µg/mL streptomycin) (BioIdea, Iran) in a humidified atmosphere at 37 ˚C with 5% $CO_2$. The cells were fed 3 times a week, and subculturing was performed when they reached 80% confluency. They were seeded at a density of 250,000 cells per well in a 6-well plate and allowed to adhere to the plate for 24 h.

### Preparation of *L. caspica* extract

The *L. caspica* algae was collected from the southern coast of the Caspian Sea (Noor, Mazandaran, Iran) and stored at -20 ˚C following freeze-drying. The dried powder was mixed with 70% ethanol and incubated for 24 h at 25 ˚C with occasional stirring. The ethanol solvent was completely removed, and these steps were repeated three times. The solvent was then totally filtered out and evaporated using a vacuum rotary evaporator at 35 ˚C. The crude extract was placed in a freeze dryer for complete removal of the solvent. The final extract was stored in the dark in a tightly closed container at 4 ˚C until use.

### Cytotoxicity assessment

Cell viability was examined using the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) assay with an MTT assay kit (Bioidea, Iran) following exposure to diazinon or *L. caspica* extract. Cells were seeded in a 96-well plate (5,000 cells per well) and were incubated for 48 h to allow them to adhere to the plate. Diazinon 98% (Ariashimi, Iran) was initially dissolved in DMSO (Dimethyl sulfoxide) to a final concentration of 0.1% and then diluted in the culture medium to obtain concentrations of 0, 25, 50, 100, 200, 400, 600, and 800 µM. Similarly, *L. caspica* extract was prepared at concentrations of 0, 25, 50, 100, 200, 400, 800, and 900 µg/mL to assess its cytotoxic potential in AGS cells. A 0.1% dimethyl sulfoxide (DMSO) solution was used as the negative control treatment. AGS cells were treated for 24 h under serum-starved conditions, and incubation was continued for an additional 4 h after treatment with 0.5 mg/mL MTT. Cell viability was estimated based on the optical density of the chromogenic purple formazan, which was measured using an ELISA reader (Bio-Rad, US) at 570 nm.

### Treatments of AGS cells with diazinon and *L. caspica* extract

Concentration ranges of diazinon and *L. caspica* extract were applied to AGS cells based on the data from cytotoxicity assay and/or previous data [4, 8]. Cells were seeded in a 6-well plate at a density of 6000 cells per well and incubated for 48 h to allow them to attach to the bottom of the plate. They were then incubated with diazinon at concentrations of 0.1, 0.5, 1, 5, and 10 µM and with *L. caspica* extract at concentrations of 10, 20, 30, and 40 µg/mL for 24 h. To assess the effect of circadian time, the treatment of diazinon was performed at three different time points: 8:00±1:00 am, 2:00±1:00 pm, and 8:00±1:00 pm. Additionally, we conducted a co-treatment assay involving 24 h of incubation with 0.1 µM diazinon following pretreatment with the above-mentioned concentrations of *L. caspica* extract to assay its protective efficacy against diazinon-induced transcriptional dysregulation of *CYP2D6*.

### Quantitative real-time PCR analysis of *CYP2D6* transcription

Total RNA was isolated from AGS cells using the Qiagen RNeasy Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. cDNA was synthesized from 1 µg of total RNA using the Multiscribe Reverse Transcriptase enzyme (Thermo- Fisher). Quantitative polymerase chain reaction (qPCR) amplification of *CYP2D6* was performed using an ABI 7900 real-time PCR system and the QuantiNova SYBR Green PCR Kit (Qiagen, Hilden, Germany), along with a *specific* primer pair (Table 1) designed based on the CYP2D6 mRNA sequence (accession number AC254996.1) available from GenBank. The β-actin primer pair (Table 1) was also designed based on the *ACTB* mRNA sequence (accession number NM_001101.5). β-actin was used as the internal control for data normalizations. Relative gene expression was quantified using the $2^{-\Delta\Delta CT}$ method. qRT-PCR were carried out three times, with each sample analyzed in triplicate, and the results are expressed as the Mean±SD.

### Statistical analysis

Statistical analysis was performed using SPSS software, version 25.0 (IBM, USA). Differences among the means for the various treatments were analyzed using one-way ANOVA [31]. P-values from multiple comparisons were adjusted using Bonferroni's multiple-comparisons test following analysis of variance (ANOVA). The effects of different circadian times were evaluated using ordinary tow-way ANOVA followed by Šidák's multiple comparisons test on log2-transformed ΔCT values. A $P<0.05$ was considered statistically significant.

**Table 1.** Primer pairs used for quantitative real-time PCR of CYP2D6

| Primer | Sequence | Amplicon Size (bp) |
|---|---|---|
| CYP2D6-F | 5′ GCTCTTCCTCTTCTTCACCTCC 3′ | 102 bp |
| CYP2D6-R | 5′ CTCACCAGGAAAGCAAAGACAC 3′ | |
| β-actin-F | 5′ CACCATTGGCAATGAGCGGTTC 3′ | 135 bp |
| β-actin-R | 5′ AGGTCTTTGCGGATGTCCACGT 3′ | |

## Results

### Cytotoxicity assessment

The MTT assay results indicated slight cytotoxicity at concentrations below 25 µM. However, cell viability was drastically reduced at concentrations over 30 µM, and the cells eventually all died at a concentration of 150 µM or higher. An $IC_{50}$ value of 80 µM was recorded for diazinon in AGS cells (Figure 1). Similarly, the cytotoxicity of *L. caspica* was evaluated following treatment with 0-900 µg/mL of its ethanol extract (Figure 1). Interestingly, the data revealed growth-promoting potential of the extract at concentrations up to 70 µg/mL, beyond which the cells gradually began to die. An $IC_{50}$ value of 840 µg/mL was recorded for this treatment. Gas chromatography-mass spectroscopy (GC-MS) analysis of the compounds was carried out on the ethanolic dried-galls extract of *L. caspica*. Twenty-five different compounds were found in the extract. The most abundant individual constituents and their approximate percentages included terpenoids and polyphenols, such as cholesterol (20.76%), phytol (3.09%), pentadecanal (4.4%), tetradecanoic acid (2.43%), tridecanedial (9.48%) and bicyclo[10.1.0]tridec-1-ene (4.87%).

### Quantitative real-time PCR analysis of *CYP2D6* transcription

*CYP2D6* mRNA levels were quantified in AGS cells using qRT-PCR following treatment with either diazinon or *L. caspica* extract, as well as following cotreatment with both. The amplification plot revealed differential *CYP2D6* expression following the various treatments, and the melting curves showed no non-specific bands or secondary structures (Figures 2A and 2B). An intense band of the expected size, without primer-dimers, was generated following 40 rounds of real-time PCR amplification of the CYP2D6 and β-actin cDNAs (Figure 2C).

Data from the diazinon treatments revealed transcriptional alterations in *CYP2D6* among the various concentrations (Table 2). However, irregular transcription rates were recorded when the mRNA levels from each of the

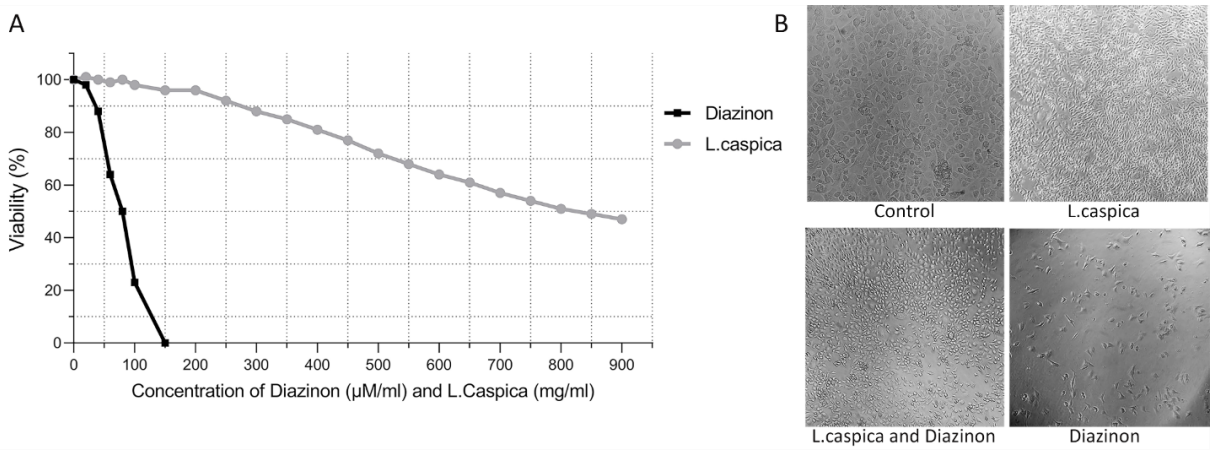



**Figure 1.** Cytotoxicity assessment

A) Viability of AGS cells after 24 h of exposure to various concentrations of diazinon (0, 25, 50, 100, 200, 400, 600, and 800 µM) and *L. caspica* extract (0, 25, 50, 100, 200, 400, 800, and 900 µg/mL): The x-axis values are shown on a logarithmic scale for better visualization; B) Morphological changes in AGS cells (control) following treatment with *L. caspica* extract, diazinon, and the combination of both (*L. caspica* + diazinon)

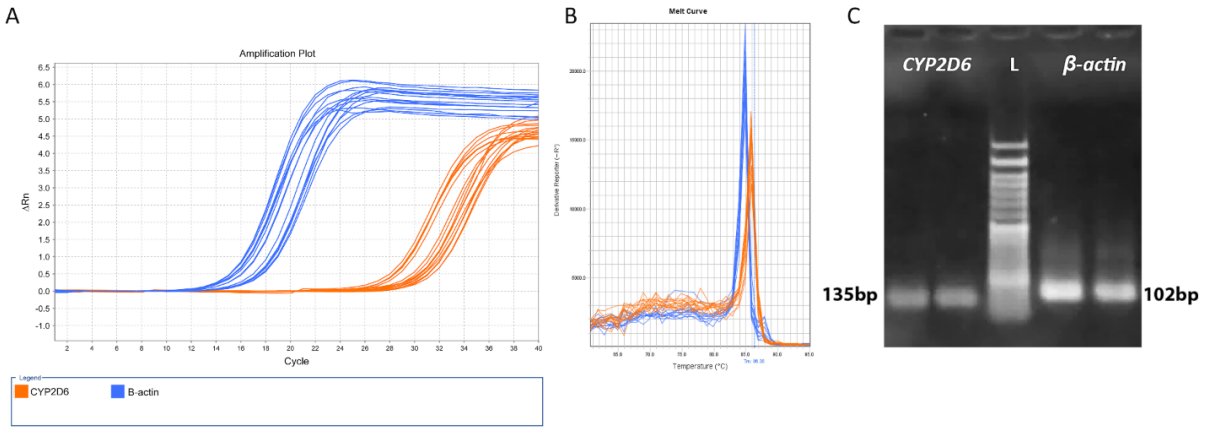



**Figure 2.** qRT-PCR

A) Amplification curves of CYP2D6 and β-actin (internal control) cDNAs plotted based on CT values versus fluorescent intensities; B) Melting curves plotted based on - ΔF/ΔT versus temperature, showing specific amplification of CYP2D6 and β-actin cDNAs using real-time PCR, without primer dimers and non-specific products; C) Agarose gel electrophoresis of real-time PCR-amplified CYP2D6 and β-Actin cDNAs on a 1.5% agarose gel [L: 100 bp ladder (fermentas)]

three independent experiments were compared (Figure 3A, 3B and 3C). Nevertheless, the mean of all the data produced a bell-shaped curve, in which the 0.1 and 10 µM treatments caused the greatest downregulation of *CYP2D6*, whereas its transcription remained unchanged following treatment with 1 µM diazinon (Figure 3D). Statistical analyses revealed a significant difference between the mRNA level at the 0.1 µM concentration and that of the normal control treatment (p_adj =0.02) (Table 2).

*CYP2D6* transcription was also evaluated following treatment of the AGS cells with 0-40 µg/mL of *L. caspica* extract (Table 3). Data showed significant upregulation of *CYP2D6*, particularly at higher extract concentrations. Specifically, a fourfold increase in mRNA levels was observed with the 40 µg/mL extract concentration (p_adj <0.001) (Figure 3E). *CYP2D6* transcription was also evaluated following treatment of AGS cells with diazinon (0.1 µM) after pretreatment with 0 (DMSO), 10, 20, 30, and 40 µg/mL of *L. caspica* extract (Table 4). Similarly, in the cotreatment assay, which included treatment of cells pretreated with 0–40 µg/mL of *L. caspica* extract with 0.1 µg/mL diazinon, mRNA levels increased in a dose-dependent manner, especially at higher concentrations (Figure 3F).

## Discussion

*CYP2D6* transcription alterations were evaluated in AGS cells following treatment with either the diazinon pesticide or *L. caspica* extract. Additionally, a co-treatment assay with both diazinon and *L. caspica* extract

**Table 2.** Mean mRNA levels following treatment with 0 (DMSO), 0.1, 0.5, 1, 5, and 10 µM diazinon and the effects of different circadian times

| Treatments | Mean $\Delta\Delta^{CT}$±SE | Adjusted P[a] | | | | | P[b] |
|---|---|---|---|---|---|---|---|
| | | DMSO | 0.1 µM | 0.5 µM | 1 µM | 5 µM | |
| DMSO | 1 | - | | | | | 0.0021** |
| 0.1 µM | 0.572±0.286 | 0.002 | - | | | | 0.0019** |
| 0.5 µM | 0.788±0.541 | 0.123 | 0.096 | - | | | 0.00011*** |
| 1 µM | 0.984±0.759 | >0.999 | 0.0028 | 0.368 | - | | 0.0428* |
| 5 µM | 0.782±0.453 | 0.105 | 0.113 | >0.999 | 0.317 | - | 0.00012*** |
| 10 µM | 0.707±0.431 | 0.202 | 0.55 | >0.999 | 0.062 | >0.999 | 0.00162** |

[a]Bonferroni's multiple-comparisons test, [b]Šidák's multiple-comparisons test, *P<0.05, **P<0.01, ***P<0.001.

**Table 3.** *CYP2D6* mRNA levels following treatment with *L. caspica* extract

| Treatments | Mean $\Delta\Delta^{CT}$±SE | Adjusted P* | | | | |
|---|---|---|---|---|---|---|
| | | DMSO | 10 µg | 20 µg | 30 µg | 40 µg |
| DMSO | 1 | - | | | | |
| 10 µg | 1.598±0.223 | 0.004 | - | | | |
| 20 µg | 1.658±0.22 | 0.002 | >0.99 | - | | |
| 30 µg | 3.47±0.257 | <0.001 | <0.001 | <0.001 | - | |
| 40 µg | 3.813±0.246 | <0.001 | <0.001 | <0.001 | 0.15 | - |

*α=0.05.

Note: Number of comparisons per family =4

was included in the study in an attempt to rectify diazinon-induced dysregulation of *CYP2D6*. The results of diazinon treatment, carried out as three independent tests in triplicate, revealed transcriptional variation among the three independent experiments.

Various physiological and behavioral activities, such as the metabolism of the exogenous compounds are subject to circadian rhythm oscillations that occur in various tissues, like the brain and peripheral tissues, including the liver, kidney, heart, skin, and lungs [32, 33]. Previous in vitro studies using cultured cells, such as Rat-1 fibroblasts and HepG2 cells, and in vivo studies in mice confirmed the rhythmic expression of some drug-metabolizing *CYP* genes, including *CYP2D6*. In particular, *CYP2D6* mRNA oscillates over time in serum-shocked (or synchronized) HepG2 cells [34-36]. These results show the dosing-time-dependent efficacy of many drugs and xenobiotics. Thus, in addition to interindividual variation resulting from its high sequence polymorphism, CYP2D6 exhibits high intraindividual variation due to oscillations in its circadian clock [36].

The mean data from the three independent tests and the resulting plot represent a bell-shaped curve, in which the 0.1 and 10 µM treatments at the extremes caused the greatest downregulation of *CYP2D6*, whereas its transcription remained almost unchanged following treatment with 1 µM diazinon. However, statistical analyses showed that only the 0.1 µM diazinon treatment caused a significant difference compared with the healthy con trol treatment. The downregulation of *CYP2D6* might be linked t to cellular stress or to the inhibitory role of diazinon in preventing CYP enzymes from carrying out their drug-metabolizing functions, which, in turn, may negatively affect their transcription [37].

Treatment of the cells with *L. caspica* extract caused clear, dose-dependent upregulation of *CYP2D6*, resulting in a 4-fold increase in mRNA levels following treatment with 40 µg/mL of the extract. Despite significant

**Table 4.** *CYP2D6* mRNA levels following treatment with diazinon (0.1 µM) after pre-treatment with 0 (DMSO), 10, 20, 30, and 40 µg of *L. caspica* extract

| Treatments | Mean $\Delta\Delta^{CT}$±SE | Adjusted P* | | | | | |
|---|---|---|---|---|---|---|---|
| | | $C^-$ | $C^+$ | 10 µg | 20 µg | 30 µg | 40 µg |
| $C^-$ | 1 | - | | | | | |
| $C^+$ | 0.68±0.02 | 0.026 | - | | | | |
| 10 µg | 1.29±0.18 | 0.552 | 0.068 | - | | | |
| 20 µg | 1.52±0.4 | 0.032 | <0.001 | 0.031 | - | | |
| 30 µg | 2.81±0.1 | <0.001 | <0.001 | <0.001 | <0.001 | - | |
| 40 µg | 3.38±0.17 | <0.001 | <0.001 | <0.001 | <0.001 | 0.0021 | - |

*α=0.05.

Note: Number of comparisons per family=5, $C^+$: Positive control (diazinon 0.1 µM), $C^-$: Negative control (DMSO).

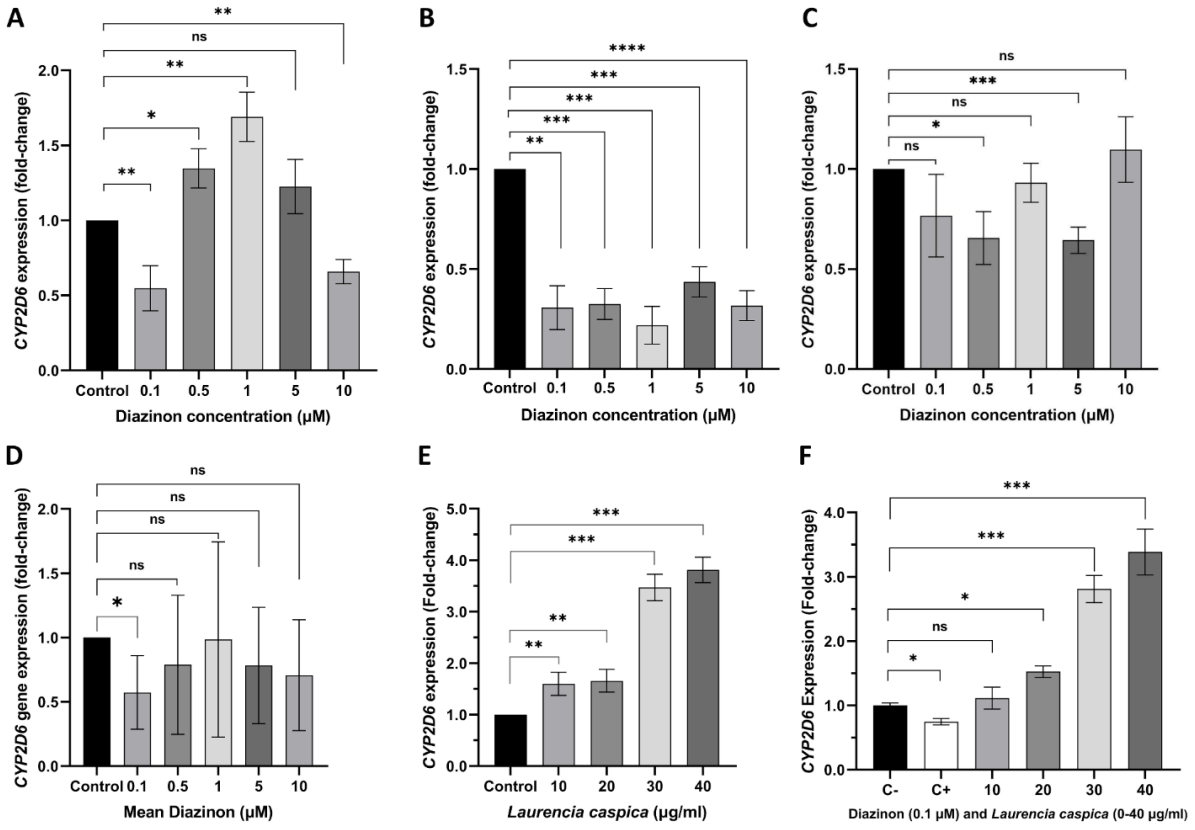



**Figure 3.** mRNA levels of CYP2D6 in AGS cells estimated by quantitative real-time PCR analysis following treatment with diazinon, *L. caspica* extract, and their combination

A- C) Three independent qPCR analyses of *CYP2D6* mRNA levels following treatment with 0 (DMSO), 0.1, 0.5, 1, 5, and 10 μM diazinon for 24 h at three different circadian times; D) Mean results from the three individual qPCR experiments; E) *CYP2D6* mRNA levels following treatment with 0 (DMSO), 10, 20, 30, and 40 μg/mL *L. caspica* extract for 24 h; F) *CYP2D6* transcription following treatment with 0.1 μM diazinon for 24 h in cells pretreated with 0 (DMSO), 10, 20, 30, and 40 μg/mL *L. caspica* extract for 24 h.

*P≤0.05, **P≤0.01, ***P≤0.001.

Note: The lines connecting the columns and the asterisks above them show significant differences between the treatment means.

interindividual variation in CYP2D6 activity, it is known that *CYP2D6* is not inducible by xenobiotics, although a limited number of the exogenous compounds have been proposed to activate *CYP2D6* expression [38]. The 4-fold upregulation observed in the present study suggests that *L. caspica* may be a potent inducer of *CYP2D6* transcription. Bioactive molecules associated with fewer side effects offer more advantages than synthetic compounds. One of the primary sources of these natural products is the marine environment. In the field of cancer research, the potential of marine algae as a source of anticancer products has been studied primarily using crude extracts or partially purified fractions [39].

A large number of secondary metabolites, including alkaloids, diterpenes, triterpenes, and polyphenolic compounds, are mainly produced by red macroalgae of the genus *Laurencia* in the marine environment [40, 41]. We recently isolated and differentiated bioactive molecules from *L. caspica* using GC–MS. The analysis showed the presence of 25 compounds, some of which belong to the terpene and polyphenol classes. These compounds could be involved in mechanisms regulating gene expression, leading to the upregulation or downregulation of transcription [42].

When cells pretreated with *L. caspica* extract were treated with diazinon, a transcriptional pattern similar to that observed with *L. caspica* extract alone was observed, implying that the extract could regulate the transcriptional oscillations induced by exposure to the diazinon pesticide. In vitro and animal studies have suggested

that diazinon induces carcinogenicity mainly through epigenetic alterations in the regulatory regions of xenobiotic-metabolizing genes [43-45]. In this context, *CYP2D6* expression can be strongly inhibited by DNA hypermethylation, histone modifications, or differential expression of transcription factors or microRNAs, all of which may be affected by diazinon treatment [34, 46].

The results of the present study show that bioactive molecules from *L. caspica* have antioxidant and proliferative activities at lower concentrations, in addition to exerting a strong stimulatory effect on *CYP2D6* transcription. Moreover, they could suppress mRNA oscillations and stably increase mRNA levels. Further research is needed to fractionate the compounds and study their effects separately.

## Conclusion

Despite comprising only a small proportion of total hepatic CYP (1–2%), CYP2D6 metabolizes the second-highest number of clinical drugs in hepatic and extrahepatic tissues. The absence of biological inducers and, to a lesser extent, clinical inducers, along with the high inter-individual variability in *CYP2D6* expression, has made its study challenging. We used an ethanolic extract of *L. capsica* as a potent inducer of *CYP2D6* transcription and to compensate for diazinon-induced *CYP2D6* dysregulation. *L. caspica* at concentrations of up to 70 µg/mL enhanced cell viability, indicating antioxidant and proliferative activities. The fourfold transcriptional upregulation of *CYP2D6* following treatment with *L. caspica* suggests that it could be a strong biological inducer of *CYP2D6*. The *L. caspica* extract could both overcome diazinon-induced *CYP2D6* dysregulation and stably increase its mRNA level. These features may indicate that bioactive molecules from *L. caspica* are plausible candidates for detoxifying clinical chemotherapy drugs through CYP2D6 hyperactivatio. However, additional research is needed to support this hypothesis.

## Ethical Considerations

### Compliance with ethical guidelines

This article does not involve human or animal samples.

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### Author's contributions

Conceptualization, methodology, formal analysis, and writing the original draft: Ali Barzegar and Shima Yahoo-Torghabe; Supervision: Zahra Hosseini-Khah; Resources: Zahra Hosseini-Khah and Ali Pakdin-Parizi; Final approval: All authors.

### Conflict of interest

The authors declared no conflict of interest.

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