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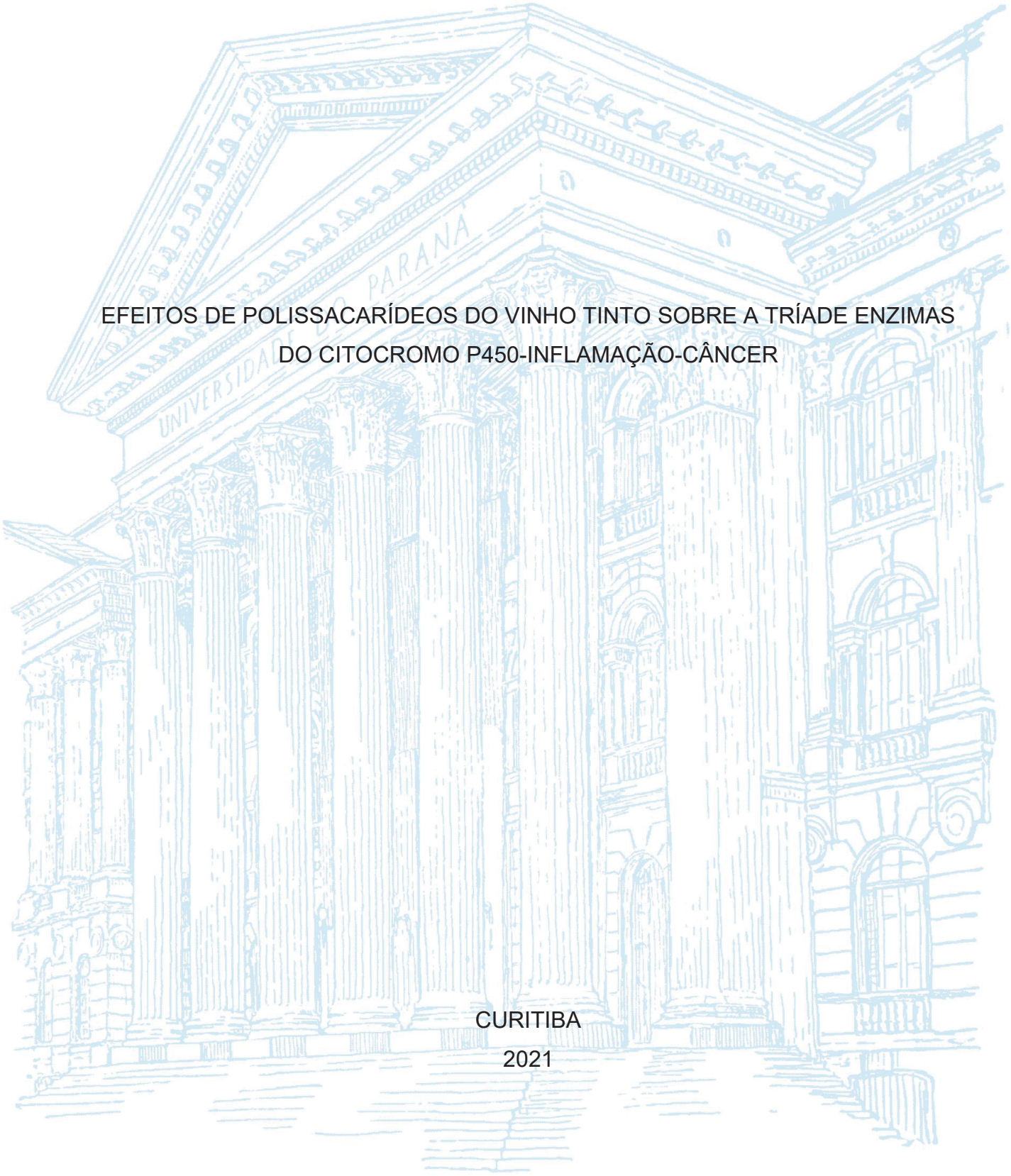

EFEITOS DE POLISSACARÍDEOS DO VINHO TINTO SOBRE A TRÍADE ENZIMAS DO CITOCROMO P450-INFLAMAÇÃO-CÂNCER

CURITIBA
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EFEITOS DE POLISSACARÍDEOS DO VINHO TINTO SOBRE A TRÍADE ENZIMAS DO CITOCROMO P450-INFLAMAÇÃO-CÂNCER

Tese apresentada ao curso de Pós-Graduação em Farmacologia, Setor de Ciências Biológicas, Universidade Federal do Paraná, como requisito parcial à obtenção do título de Doutora em Farmacologia

Orientadora: Profa. Dra. Alexandra Acco

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Lute com determinação, abrace a vida com paixão,
perca com classe e vença com ousadia,
porque o mundo pertence a quem se atreve e
a vida é muito para ser insignificante.
(Branco, Augusto)

RESUMO

O microambiente tumoral é composto, além das células tumorais, por células do sistema imunológico, desencadeando um processo inflamatório crônico, onde ocorre liberação de citocinas pró-inflamatórias, levando a uma importante modulação do sistema citocromo P450 (CYP), principalmente no tecido hepático. Considerando essa regulação, avaliamos os efeitos da fração solúvel de polissacarídeos extraídos do vinho tinto cabernet franc (SFP) sobre a tríade citocromo P450-inflamação-câncer, uma vez que seus efeitos antitumorais ocorrem através do aumento de TNF-α no tecido tumoral e modulação imunológica. Utilizamos células HepG2 em cultivo para análises dos efeitos da SFP in vitro, onde verificamos a citotoxicidade e expressão gênica; e o modelo de tumor Walker-256 em ratos submetidos a três protocolos: *a)* protocolo de tumor sólido, com animais portadores de tumor sólido e livres de tumor (Basal), tratados com 60 mg/kg da SFP isolada ou associada a 0,5 mg/kg de vincristina, por 14 dias; *b)* protocolo de tumor líquido, com animais portadores do tumor ascítico , tratados com 60 mg/kg da SFP ou 0,5 mg/kg de vincristina, por 5 dias; *c)* protocolo de quimioprevenção, com animais portadores ou não de tumor sólido, tratados com 6 mg/kg de SFP por 14 dias antes da inoculação, e com 6 mg/kg da SFP, isolada ou associada a 0,5 mg/kg de vincristina, por 14 dias após a inoculação do tumor. Nos protocolos *a* e *c*, peso e volume tumoral foram avaliados, enquanto em *b* o volume ascítico e o número de células tumorais viáveis foram mensurados. Após a eutanásia, o sangue dos animais foi encaminhado para análises hematológicas e bioquímicas, e o fígado foi utilizado para as análises de CYP total, atividade enzimática de mieloperoxidase (MPO) e N-acetilglucosaminidase (NAG) e determinação dos níveis de TNF-α; assim como para expressão gênica, juntamente com tecido intestinal. Os resultados in vitro demonstram inibição da *CYP1A2* em todos os grupos tratados com a SFP, sem redução da viabilidade das células HepG2 pela SFP isolada. Os dados hepáticos demonstraram que os níveis de CYP total diminuíram no grupo veículo em comparação com o naive, e em todos os grupos tratados com a SFP (com e sem tumor) e vincristina, nos protocolos de tumor sólido e líquido, enquanto houve aumento da atividade de NAG e dos níveis de TNF-α para esses grupos, no protocolo de tumor sólido. Neste protocolo, a SFP também inibiu a expressão das enzimas *Cyp3a9*, *Cyp2e1* e *Cyp1a1* no tecido hepático. De modo interessante, o protocolo de quimioprevenção não alterou os níveis de CYP total nos tecidos hepático e intestinal, nem os parâmetros inflamatórios hepáticos avaliados. A fim de investigar se a SFP chegaria intacta ao intestino, realizamos uma análise de digestão in vitro, onde identificamos que a SFP ficou praticamente intacta, sem ser degradada. Dessa forma, conclui-se que os efeitos observados são resultado da ação da SFP íntegra, na dose mais alta testada, que leva a uma importante modulação imunológica no tecido hepático, resultando na inibição de CYPs, especialmente, das *Cyp1a1*, *Cyp2e1* e *CYP1A2*, que estão envolvidas no processo de carcinogênese. Esses conhecimentos fornecem uma melhor compreensão da via de inflamação e de câncer na inibição de CYPs, do papel da SFP ao desencadear essa via, e do importante efeito antitumoral da SFP através da inibição da subfamília CYP1A.

Palavras-chave: Citocromo p450. Inflamação. Câncer. Tumor Walker-256. TNF-α.

ABSTRACT

The tumor microenvironment is composed of tumor cells and cells of the immune system, triggering a chronic inflammatory process, where pro-inflammatory cytokines are released, leading to an important modulation of the cytochrome P450 (CYP) system, mainly in the liver tissue. Considering this regulation, we evaluated the effects of the soluble polysaccharide fraction extracted from cabernet franc red wine (SFP) on the cytochrome P450-inflammation-cancer triad, since its antitumor effects are related to the increase of TNF-α in tumor tissue and immune modulation. Cultured HepG2 cells were used for analyses of the effects of SFP in vitro, where cytotoxicity and gene expression were evaluated; and the Walker-256 tumor model in rats subjected to three protocols: *a)* solid tumor protocol, with animals bearing solid tumor, and tumor free (Basal), treated with 60 mg/kg of SFP alone or associated with 0.5 mg/kg of vincristine, for 14 days; *b)* liquid tumor protocol, with animals bearing ascitic tumor, treated with 60 mg/kg of SFP or 0.5 mg/kg of vincristine, for 5 days; *c)* chemoprevention protocol, with animals with or without solid tumor, treated with 6 mg/kg of SFP for 14 days before inoculation, and with 6 mg/kg of SFP, alone or associated with 0.5 mg/kg of vincristine, for 14 days after tumor inoculation. In protocols *a* and *c*, the tumor weight and tumor volume were evaluated, while in *b* ascitic volume and the number of viable tumor cells were measured. After euthanasia, blood from the animals was applied in hematological and biochemical analyses, and the liver was used for total CYP, myeloperoxidase (MPO) and N-acetylglucosaminedase (NAG) enzyme activity, and determination of TNF-α levels; as well as for gene expression, along with intestinal tissue. The in vitro results demonstrate inhibition of *CYP1A2* in all groups treated with SFP, with no reduction in viability of HepG2 cells by SFP alone. Hepatic data demonstrated that total CYP levels decreased in the vehicle group compared to the naive group, and also in all groups treated with SFP (with and without tumor) and vincristine in the solid and liquid tumor protocols, while there was increased NAG activity and TNF-α levels for these groups in the solid tumor protocol. In this protocol, SFP also inhibited the expression of *Cyp3a9*, *Cyp2e1* and *Cyp1a1* enzymes in liver tissue. Interestingly, the chemoprevention protocol did not alter total CYP levels in liver and intestinal tissues, nor did it alter the liver inflammatory parameters evaluated. In order to investigate whether SFP would reach the intestine intact, we performed an in vitro digestion analysis, where we identified that SFP was almost intact, without being degraded. Thus, we conclude that the observed effects are a result of the action of intact SFP, in the highest dose tested, which leads to an important immune modulation in liver tissue, resulting in the inhibition of CYPs, especially *Cyp1a1, Cyp2e1* and *CYP1A2*, which are involved in the process of carcinogenesis. This knowledge provides a better understanding of the inflammation and cancer pathway in inhibiting CYPs, the role of SFP in triggering this pathway, and the important antitumor effect of SFP through inhibition of the CYP1A subfamily.

Keywords: Cytochrome p450. Inflammation. Cancer. Walker-256 tumor. TNF-α.

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LISTA DE ABREVIATURAS OU SIGLAS

INTRODUÇÃO

CTLA-4 - Antígeno 4 associado ao linfócito T citotóxico
CYP450 - Citocromo P450
IL-1 - Interleucina-1
IL-6 - Interleucina-6
IFN-γ - Interferon-gama
PD-1 - Morte programada 1
TAMs - Macrófagos associados ao tumor
TNF-α - Fator de necrose tumoral-alfa

ARTIGO CIENTÍFICO 1 – "INVOLVEMENT OF CYTOCHROME P450 ENZYMES IN INFLAMMATION AND CANCER: A REVIEW"

4-OHCP - 4-hydroxy-cyclophosphamide
20-HETE - Hydroxyeicosatetranoic acid
AC - Doxorubicin and cyclophosphamide
AhR - Aryl hydrocarbon receptor
ARNT - Aryl hydrocarbon receptor nuclear translocator
CAR - Constitutive androstane receptor
CYP450 - Cytochrome P450
DNA - Deoxyribonucleic acid
E2 - Estradiol
EET - 4-epoxyeicosatrienoic
ERs - Estrogen receptors
ERCC1 - Repair cross-complementary 1
HCC - Liver hepatocellular carcinoma
HCV - Hepatitis C virus
HBV - Hepatitis B virus
Hcy - Homocysteine
HHcy - Hyperhomocysteinemia
IL-1 - Interleukin-1
IL-6 - Interleukin-6

IFN-γ - Interferon-γ
K_i - Inhibitory constants
LPS - Lipopolysaccharide
LTA - Lipotechoic acid
Mrna - Messenger ribonucleic acid
Mrp2 - Multidrug resistance-associated protein 2
PXR - Pregnane X receptor
Sultn - Amine N-sulfotransferase
TGF-β - Transforming growth factor β
TNF-α - Tumor necrosis factor α
Ugt1a1 - UDP glucuronosyltransferase family 1 member A1
V_{max} - Maximal metabolic velocity
XREs - Xenobiotic response elements

ARTIGO CIENTÍFICO 2 – "INFLUENCE OF RED WINE POLYSACCHARIDES ON CYP ENZYMES AND INFLAMMATORY PARAMETERS IN TUMOR MODELS"

ALT - Alanine aminotransferase
ANOVA - Analysis of variance
AST - Aspartate aminotransferase
Cis - Cisplatin
CS - Ciplatin + SFP
CV - Cisplatin + Vincristine
CVS - Cisplatin + Vincristine + SFP
CYP - Cytochrome P450
D_2O - Deuterium oxide
EDTA - Ethylenediaminetetraacetic acid
ELISA - Enzyme linked immunosorbent assay
FBS - Fetal bovine serum
GAPDH - Glyceraldehyde 3-phosphate dehydrogenase
HCC - Liver hepatocellular carcinoma
IL-1 - Interleukin-1
IL-6 - Interleukin-6
INFs - Inferferons

i.p.	- Intraperitoneal
MPO	- Myeloperoxidase
NAG	- N-acetylglucosaminidase
PAMPs	- Pathogen-associated molecular patterns
PBS	- Phosphate buffer
qPCR	- Quantitative polymerase chain reaction
SEM	- Standard error of the mean
SFP	- Soluble fraction of polysaccharides from cabernet franc red wine
s.c	- Subcutaneously
TLRs	- Toll-like receptors
TMSP	- Trimethylsilyl propionic acid
TNF-α	- Tumor necrosis factor-alpha
Veh	- Vehicle
Vin	- Vincristine
VS	- Vincristine + SFP

SUMÁRIO

1 INTRODUÇÃO

1.1 CITOCROMO P450 (CYP) E INFLAMAÇÃO

As enzimas do citocromo p450 (CYP) correspondem a uma superfamília de heme-proteínas, determinadas pela sua complexidade e capacidade metabólica. Até o momento, 57 enzimas CYP apresentam sua função devidamente identificada, das quais seis (CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP2E1 e CYP3A4) estão diretamente ligadas ao metabolismo de 90% dos medicamentos (STAVROPOULOU, PIRCALABIORU, BEZITZOGLOU, 2018). Além do metabolismo de xenobióticos, as CYP possuem funções como síntese de hormônios, regulação de ácidos graxos, assim como metabolismo de vitaminas lipossolúveis e hormônios (BERNHARDT, 2006). De modo geral, sua localização é predominantemente hepática, mas estão presentes em outros órgãos como no intestino e nos rins (GILANI, CASSAGNOL, 2021; STAVROPOULOU, PIRCALABIORU, BEZITZOGLOU, 2018).

Considerando seu efeito primordial sobre o metabolismo de fármacos, esse grupo de enzimas pode estar ligado a grande parte das reações adversas ou ainda falha terapêutica encontradas na prática clínica. Essas respostas diversas estão relacionadas a variações genéticas das CYPs, e/ou a fatores não genéticos (ZANGER, SCHWAB, 2013). Um importante fator não genético é o processo inflamatório, que promove uma regulação negativa sobre essas enzimas (SHAH, SMITH, 2015). Contudo, esse efeito depende das características dos pacientes (idade, função renal, uso de medicamentos), da variabilidade genética, do grau da inflamação, do perfil de citocinas e da via metabólica de um fármaco. As principais citocinas responsáveis por esse efeito são o TNF-α (fator de necrose tumoral alfa), a IL (interleucina)-1 e IL-6. Portanto, para otimizar um tratamento personalizado, torna-se necessário compreender como o processo inflamatório compromete a farmacocinética, de modo especifico, no metabolismo dos fármacos (DE JONG et al., 2020).

1.2 RELAÇÃO ENTRE CÂNCER E O PROCESSO INFLAMATÓRIO

A inflamação está diretamente associada ao desenvolvimento das células neoplásicas, que promovem modificações na composição e na função do sistema

imunológico, contribuindo para a sobrevivência, proliferação e disseminação destas células (CARVALHO et al., 2016; HIAM-GALVEZ; ALLEN; SPITZER, 2021). De modo geral, a imunidade é regulada através de interações entre as diferentes linhagens celulares e seus tecidos, sendo que no microambiente tumoral (HIAM-GALVEZ; ALLEN; SPITZER, 2021), as células imunes encontram um ambiente rico em nutrientes e oxigênio, e composto por células cancerosas, células-tronco tumorais, células endoteliais e estromais, fibroblastos associados ao tumor e células do sistema imunológico, como os macrófagos associados ao tumor (TAMs). Os TAMs são as principais células envolvidas no processo inflamatório e atuam como promotor tumoral (CARVALHO et al., 2016; HANAHAN; WEINBERG, 2011; MANTOVANI et al., 2017; JUNTTILA; DE SAUVAGE, 2013).

Devido à presença de células inflamatórias, as citocinas pró-inflamatórias estão presentes no microambiente tumoral, e são responsáveis pelos efeitos mutagênicos. As principais citocinas envolvidas no desenvolvimento tumoral são o TNF-α, IL-1e IL-6. O TNF-α é a principal citocina pró-inflamatória e está envolvido em todos os aspectos da carcinogênese, como na transformação celular, sobrevivência, proliferação, invasão, angiogênese e metástase (WANG; LIN, 2008). Muitos modelos tumorais malignos são capazes de produzir TNF-α em pequenas quantidades, melhorando o crescimento e a expressão de genes carcinogênicos (BALWILL, 2009).

Além de participar da progressão tumoral, um processo inflamatório crônico aumenta o risco de desenvolvimento de vários tipos de cânceres, como os pulmonares, gástricos, cervicais, colorretais, bem como de hepatocarcinoma, mieloma e linfoma de Hodgkin (ELINAV et al., 2013; GRIVENNIKOV, GRETEN, KARIN, 2011).

A regulação do microambiente inflamatório tumoral também está associada a mutações nas células tumorais que originam antígenos reconhecidos pelos linfócitos T através de moléculas “checkpoint”, tal como PD-1 (morte programada 1) e CTLA-4 (antígeno 4 associado ao linfócito T citotóxico). Esses são conhecidos como pontos de verificação imunológica, e regulam negativamente a função dos linfócitos T. De modo geral, essas células também estão diminuídas no microambiente tumoral e possuem déficits funcionais. Para burlar esses mecanismos, atualmente tem se utilizado a imunoterapia, que através dos inibidores dos checkpoints imunológicos promove um aumento da ativação do sistema

imunológico. Os inibidores de checkpoint aprovados até o momento para a terapia do câncer são o inibidor do CTLA-4 (ipilimumab), utilizado no tratamento do melanoma, e os inibidores da PD-1 (nivolumab e permbrolizumab) que são aplicados, na terapia do câncer de pulmão de células pequenas e no melanoma (BUCHBINDEZ; DESAI, 2016; GURUSAMY ET AL., 2017; HIAM-GALVEZ; ALLEN; SPITZER, 2021).

1.2.1 Modelo celular HepG2

A linhagem celular de hepatoma humano HepG2 corresponde a células com elevada taxa de proliferação e não tumorigênicas. São de origem epitelial, desempenhando diferentes funções hepáticas, como secreção de proteínas (albumina, α2-macroglobulina, α1-antitripsina, transferrina e plasminogênio). Essa linhagem tem sido amplamente utilizada para estudos in vitro do metabolismo de drogas e hepatotoxicidade, podendo ser empregada para avaliar as possíveis interações entre medicamentos. Apesar dos baixos níveis de expressão basal das enzimas CYP, essa linhagem celular é um modelo alternativo para avaliação da indução de CYP, principalmente, CYP1A2, CYP2B6 e CYP3A4, podendo ser útil como um modo de triagem para estudo das interações envolvendo as enzimas CYPs (CHOI, et al., 2014; DONATO; TOLOSA; GÓMEZ-LECHÓN, 2015).

1.2.2 Modelo tumoral animal – Walker-256

O carcinossarcoma Walker-256 (W256) é um modelo tumoral espécie-específico de ratos, que tem crescimento rápido e foi descoberto na Universidade Johns Hopkins – Pittsburgh, Estados Unidos, no ano 1928, pelo Dr. George Walker (EARLE, 1934; SCHEREK, 1935; GOLDACRE, SYLVEN, 1962; SIMPKINS et al., 1991).

Ratos portadores do tumor Walker-256 apresentam similaridades com pacientes humanos com cânceres severos (ACCO, BASTOS-PEREIRA, DREIFUSS, 2012; BLOOR, HAVER, 1954), estando associado à inflamação. Salles Perroud et al. (2006) avaliaram a liberação de citocinas pró-inflamatórias por células Walker-256, e identificaram elevados níveis de INF-γ, IL-12 e TGF-β produzidos pela variante A das células tumorais, assim como a produção de TNF-α. A produção e

liberação das citocinas pró-inflamatórias são capazes de modular o sistema enzimático CYP, uma vez que o INF-γ e TNF-α podem promover inibição enzimática.

Olson e Weiner (1980) e Raw (1983) demonstraram a relação entre o tumor Walker-256 e o metabolismo de enzimas do CYP450, e ambos observaram redução da atividade de CYPs no fígado de ratos portadores do tumor Walker-256. Ou seja, por si só este tumor, mesmo localizado em tecidos não hepáticos, afeta a atividade enzimática e, consequentemente, a capacidade metabólica do animal.

1.3 COMPOSTOS ANTITUMORAIS

Atualmente existem alguns tipos de tratamentos antineoplásicos que buscam burlar os mecanismos da progressão tumoral, sendo eles a cirurgia, radioterapia, quimioterapia, hormonioterapia e imunoterapia. A terapia mais convencional em pacientes com câncer é a quimioterapia, na qual são utilizados medicamentos para combater o desenvolvimento da doença (FRERES, JERUSALEM E MOONEN, 2017).

Dentre as drogas quimioterápicas mais utilizadas estão vincristina e cisplatina, que possuem diferentes mecanismos de ação (DE ALMEIRA et al., 2005), e que podem também afetar a atividade de CYPs (Tabela 1). Apesar de ser amplamente utilizada contra o câncer, a eficácia da quimioterapia é limitada pela toxicidade e pelos processos de resistência a fármacos (ROCHA, 2016), que incluem mecanismos que limitam a concentração da droga no meio intracelular, por meio de seu efluxo ou por bloqueio de processos envolvidos na ativação do composto (HOLOHAN et al., 2013). Por estas razões, novos fármacos antineoplásicos, eficientes e com poucos efeitos adversos, são constantes alvos de estudos. Desta forma, na tentativa de obter novos compostos com atividade antitumoral, é que os polissacarídeos obtêm destaque, dentre eles, a fração solúvel de polissacarídeos do vinho tinto.

Tabela 1 – Medicamentos quimioterápicos e sua relação com as enzimas do CYP.

Droga quimioterápica	**Mecanismo de ação**	**Efeito sobre enzimas CYP**
Cisplatina	Formação de adutos no DNA e apoptose (DASARI	Inibidor da CYP2E1 e CYP3A1/2 (AHMED et al.,

	E TCHOUNWOU, 2014).	2014) Indutor da CYP4A11 (LI et al., 2015)
Vincristina	Se liga a proteínas microtubulares, inibindo a formação dos microtúbulos e na dissolução do fuso mitótico (FRERES, JERUSALEM E MOONEN, 2017).	Inibidor da CYP3A4/CYP3A5 (EGBELAKIN et al., 2011)

1.3.1 Fração solúvel do polissacarídeo extraído do vinho tinto

Os polissacarídeos podem ser considerados substâncias modificadoras da resposta biológica, principalmente por promoverem modulação do sistema imunológico. Polissacarídeos de diversas fontes e diferentes estruturas possuem atividade antitumoral, dentre eles está a fração solúvel de polissacarídeos (SFP) extraídos do vinho tinto, composto principalmente por arabinogalactana (Ara), rhamnogalacturonana (Rha), ácido glicurônico (GalA), glicose (Glc), galactose (Gal), manose (Man), xilose (Xyl) e fucose (Fuc). A SFP demonstrou um importante efeito antitumoral contra o tumor Walker-256 em ratos por meio de modulação imunológica, com elevação de linfócitos circulantes e TNF-α tumoral, associada à diminuição de monócitos e neutrófilos sanguíneos, e óxido nítrico (NO), N-acetilglucosaminidase (NAG) e mieloperoxidase (MPO) no tecido tumoral. O aumento de TNF-α provavelmente é responsável pelo estímulo de necroptose, evidenciada pelo aumento da expressão gênica de *Rip-1* e *Rip-3* em animais tratados com a SFP na dose oral de 60 mg/kg (STIPP et al., 2017). Apesar desses efeitos, não se conhece a influência da SFP sobre enzimas de metabolismo, tampouco sua interferência na ação e biotransformação de outros quimioterápicos. Razão pela qual este trabalho foi realizado.

1.4 JUSTIFICATIVA

A SFP é uma mistura de polissacarídeos extraídos do vinho tinto, bebida mundialmente apreciada e consumida. Este é um dos fatores que torna o estudo de seus componentes interessante e necessário. Recentemente nosso grupo publicou um estudo sobre os efeitos antitumorais do tratamento com SFP (STIPP et al., 2017). Este composto promoveu modulação no microambiente inflamatório tumoral, assim como modulou células do sistema imune circulantes. Portanto, hipotetizamos que este efeito da SFP pode afetar a regulação de CYPs, que pelo aumento de citocinas (Ex: TNF-α) podem sofrer regulação negativa, e consequentemente, promover efeitos colaterais de outros fármacos administrados simultaneamente e metabolizados por estas enzimas. Estes efeitos da SFP poderiam interferir nas ações terapêuticas, ou favorecer efeitos adversos de outros quimioterápicos, como a vincristina. Neste trabalho investigamos se o tratamento antitumoral (profilático ou terapêutico) com a SFP, associada ou não à vincristina, pode ter efeitos na regulação das enzimas CYP. Para tanto, foi utilizado o tumor Walker-256 como modelo de estudo, pois já foi demonstrado que estas células liberam um "toxohormônio", responsável pela redução da síntese de enzimas CYPs no fígado de ratos portadores desse tumor, sendo, portanto, um modelo adequado para a presente proposta (OLSON, WEINER, 1980; RAW, 1983); bem como a linhagem celular humana HepG2 foi utilizada, por ser proveniente de tecido hepático e permitir estudos de possíveis interações medicamentosas.

1.5 OBJETIVOS

1.5.1 Objetivo geral

Investigar se o efeito modulador inflamatório da SFP pode regular o metabolismo hepático in vitro, diante das células HepG2, e in vivo, especialmente sobre as enzimas CYP3A9, CYP2E1, CYP2D4 e CYP1A1, quando usada isolada ou em combinação com a vincristina, um conhecido inibidor da enzima CYP3A4. Através disso, verificar se essa regulação pode prejudicar a ação deste agente quimioterápico ou induzir efeitos colaterais, diante do tumor Walker-256.

1.5.2 Objetivos específicos

a) Investigar o efeito in vitro, diante das células HepG2, do tratamento com SFP, de modo isolado ou associado aos quimioterápicos vincristina e cisplatina sobre as enzimas CYP1A2 e CYP2B6;
b) Avaliar o efeito antitumoral e sobre as enzimas CYPs pelo tratamento com SFP no protocolo de tumor líquido, iniciado após a inoculação intraperitoneal das células tumorais;
c) Avaliar o efeito antitumoral e sobre as enzimas CYPs pelo uso profilático de SFP no protocolo de quimioprevenção em tumor sólido, iniciado 14 dias antes e finalizado 14 dias depois da inoculação subcutânea das células tumorais inoculadas subcutaneamente;
d) Avaliar o efeito antitumoral e sobre as enzimas CYPs pelo tratamento de 14 dias com SFP associada à vincristina no protocolo de tumor sólido;
e) Avaliar, diante dos tratamentos acima:
    - Parâmetros hematológicos (células do sistema imune);
    - Parâmetros bioquímicos (AST, ALT e níveis de glicose);
    - Níveis de CYP total no tecido hepático;
    - Níveis de CYP total no tecido intestinal do protocolo de quimioprevenção em tumor sólido;
    - Atividade enzimática de mieloperoxidase (MPO) e N-acetilglicosaminidase (NAG) no tecido hepático;
    - Níveis de TNF-α no tecido hepático no protocolo de tumor sólido.
f) Avaliar o efeito sobre a expressão gênica das enzimas CYP3A9, CYP2E1, CYP2D4 e CYP1A1, do tratamento com SFP diante do protocolo de tumor sólido, iniciado após a inoculação subcutânea das células tumorais em ratos;
g) Investigar o perfil de digestão da SFP in vitro, em sistema livre de células.

NOTA EXPLICATIVA

Esta tese é apresentada em formato de artigos científicos, constando de um artigo de revisão de literatura (publicado no periódico *Cancer Chemotherapy and Pharmacology*, 2021, doi: 10.1007/s00280-020-04181-2) e de um manuscrito abordando os experimentos realizados, resultados e discussão, a ser submetido.

2 ARTIGO CIENTÍFICO 1 – "INVOLVEMENT OF CYTOCHROME P450 ENZYMES IN INFLAMMATION AND CANCER: A REVIEW"

Involvement of cytochrome P450 enzymes in inflammation and cancer: a review

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2.1 ABSTRACT

Cytochrome P450 (CYP) enzymes are responsible for the biotransformation of drugs, xenobiotics, and endogenous substances. This enzymatic activity can be modulated by intrinsic and extrinsic factors, modifying the organism's response to medications. Among the factors that are responsible for enzyme inhibition or induction is the release of proinflammatory cytokines, such as interleukin-1 (IL-1), IL-6, tumor necrosis factor α (TNF-α), and interferon-γ (IFN-γ), from macrophages, lymphocytes, and neutrophils. These cells are also present in the tumor microenvironment, participating in the development of cancer, a disease that is characterized by cellular mutations that favor cell survival and proliferation. Mutations also occur in CYP enzymes, resulting in enzymatic polymorphisms and modulation of their activity. Therefore, the inhibition or induction of CYP enzymes by proinflammatory cytokines in the tumor microenvironment can promote carcinogenesis and affect chemotherapy, resulting in adverse effects, toxicity, or therapeutic failure. This review discusses the relevance of CYPs in hepatocarcinoma, breast cancer, lung cancer, and chemotherapy by reviewing *in vitro*, *in vivo*, and clinical studies. We also discuss the importance of elucidating the relationships between inflammation, CYPs, and cancer to predict drug interactions and therapeutic efficacy.

Keywords: CYP450; enzyme; hepatocarcinoma; breast cancer; lung cancer; chemotherapy.

2.2 INTRODUCTION

The cytochrome P450 (CYP450) system comprises important metabolizing enzymes that are present in different tissues. These enzymes are found mainly in intestinal and hepatic tissues, but they are also present in the kidneys, lungs, and brain. The activity of CYP enzymes is influenced by various factors, including gender, disease states, the environment (e.g., herbicide exposure), alcohol consumption, herbal medications, and diet [1]. The expression of CYPs is modulated by cytokines during inflammation, which can result in alterations of the pharmacological effects of substances in inflammatory diseases, such as cancer, and alterations of the pharmacokinetics of prescriptions medications, such as chemotherapeutic drugs [2].

CYP enzymes play an important role in the deactivation of endogenous and exogenous substances. They are also responsible for the metabolism of procarcinogens to carcinogens. The main CYPs that are responsible for procarcinogen activation are CYP1A1, CYP1A2, CYP1B1, CYP2A6, and CYP2E1, gene polymorphisms of which are associated with a higher risk for some cancers [3]. Moreover, CYPs are associated with tumor development and progression and the activation of anticancer prodrugs and their metabolic clearance [4]. This review focuses on the involvement of CYPs in cancer, cancer-related inflammation, and chemotherapy.

2.2.1 Features and functions of the CYP450 system

The CYP system consists of a large group of hemeproteins that are located on the endoplasmic reticulum or in mitochondrial membranes [5]. The Human Genome Project sequenced 57 distinct CYP genes, consisting of 18 families [6, 7]. CYP genes are divided into two groups: endogenous CYPs (CYP family 7-51) and xenobiotic CYPs (CYP family 1-4) [8]. Endogenous CYPs are involved in the metabolism of sterols and are found in the testes, ovaries, and adrenal glands. One-third of CYPs are found in the liver and involved in the degradation of xenobiotics [7, 9].

The nomenclature of CYP450 reflects the absorption of pigment at 450 nm when they are reduced in the presence of CO [10]. The enzyme designation consists of a sequence of number-letter-number. The first number represents ≥ 40% sequence identity (e.g., CYP1 and CYP2). The suffix indicates the subfamily, based

on ≥ 55% sequence identity (e.g., CYP1A and CYP1B). The final number represents the individual P450 within the family/subfamily (e.g., CYP1A1 and CYP1A2 [9]; Fig. 1).

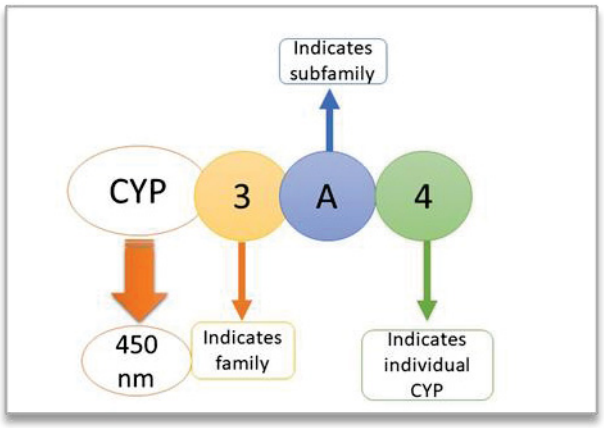

Figure 1 - CYP enzyme nomenclature.
(Figure produced by Power Point software)

CYP gene expression is regulated by the activation of many nuclear receptors, including the pregnane X receptor (PXR), constitutive androstane receptor (CAR), and aryl hydrocarbon receptor (AhR). These receptors are activated by xenobiotics [11]. CYP activity can be associated with many intrinsic and extrinsic factors that alter enzyme expression and consequently impair their function. The therapeutic response and side-effects can be different among patients who are treated with the same drug and dose, resulting in variable drug excretion and plasma drug concentrations [12]. This condition is a prerequisite for predicting pharmacokinetics and the drug response [13]. CYP1, CYP2, and CYP3 are responsible for the metabolism of 70-80% of all drugs in clinical use and other xenobiotics. The highest expressed forms of CYP in the liver are CYP3A4, CYP2C9, CYP2C8, CYP2E1. CYP1A2. CYP2A6, CYP2D6, CYP2B6, CYP2C19, and CYP3A5 are less abundant in the liver, and CYP2J2, CYP1A1, and CYP1B1 are expressed mainly extrahepatically [13]. CYP4 is involved in eicosanoid metabolism and activated by various environmental stimuli, such as diet, chemical compounds, drugs, and pheromones [7]. The expression and function of various xenobiotic CYPs are described in Table 1.

Table 1 - Examples of CYP enzymes and their function.

CYP enzyme	Organ of Expression	Function	Metabolized drugs/ compounds
CYP2A6	Lung, esophagus, nasal epithelium [14]	Arachidonic acid epoxygenase activity [15], coumarin 7 – hydroxylase activity [16], iron ion binding, steroid hydroxylase activity [15], N-nitrosomethylbenzylamine activation [17], nicotine detoxication [17, 18], tobacco-specific procarcinogen activation [18]	Cotinine, tegafur, letrozole, efavirenz, valproic acid, pilocarpine, artemisinin, artesunate, SM-12502, caffeine, tyrosol [19]
CYP1A1	Liver, placenta [20]	Estrogen 16-α hydroxylase activity [21], monooxygenase activity [15], oxidoreductase activity [22], oxygen binding [23], vitamin D 24-hydroxylase activity [24]	5-Florouracil, aminoflavone, amiodarone, bergamottin, ciprofibrate/clofibrate, curcumin, dexamethasone, ellipticine, estradiol, fluoroquinolones, flutamide, fluvoxamine, ketoconazole, propioconozole, lansoprazole, omeprazole, norfluoxetine, oltipraz, phenobarbital, quinidine, quinine, resveratrol, salicylamide, sertraline, sulindac, vitamin A [25]
CYP1A2	Liver [26]	Caffeine oxidase activity [27], demethylase activity, electron transfer activity [28], enzyme binding [29], heme binding [30], monooxygenase activity [15], oxidoredutase activity [22]	Caffeine, clozapine, theophylline [31]
CYP2C8 and CYP2C9	Liver (hepatocytes) [32]	Arachidonic acid epoxygenase activity [15], caffeine oxidase activity [27], estrogen 16-α-hydroxylase activity [33], heme binding [15], monooxygenase activity [34], steroid hydroxylase activity [15], drug binding [35]	Carvedilol, celecoxib, glipizide, ibuprofen, irbesartan, losartan [31]
CYP2D6	Liver, duodenum [36]	Drug binding [35], heme binding [15], monooxygenase activity [34], oxidoreductase activity [22], steroid hydroxylase activity [15]	Amitriptyline, carvedilol, codeine, donepezil, haloperidol, metoprolol, paroxetine, risperidone, tramadol [31]
CYP3A4	Liver, duodenum [36]	Caffeine oxidase activity [27], enzyme binding [29], estrogen 16-α-hydroxylase activity [33], iron binding [37], monooxygenase activity [38], oxidoreductase activity [22], oxygen binding [39], steroid binding [37], steroid hydroxylase activity [40], testosterone 6-β-hydroxylase activity [41], vitamin D 24-hydroxylase activity [24], vitamin D3 25-hydroxylase activity [24]	Alprazolam, amlodipine, atorvastatin, cyclosporine, diazepam, estradiol, simvastatin, sildenafil, verapamil, zolpidem [31]

Mutations of CYP genes can cause metabolism defects and contribute to several clinically relevant diseases [42]. The genetic instability of metabolic rate is

determined by genetic factors, such as monogenic polymorphisms, which may partially explain variations in only a few enzymes, such as CYP2D6. Most enzymes are multifactorially controlled, including additional polymorphisms in regulatory *trans*-genes and nongenetic host factors, including sex, age, disease state, and hormonal and diurnal influences. Environmental and behavioral factors (e.g., diet, drug co-administration, and smoking) can influence enzymatic activity [12, 13]. Thus, through interactions between environmental factors, CYP can be induced or inhibited by some drugs, resulting in the different activity or toxicity of xenobiotics. Evaluations of the effects of drugs on these enzymes are useful for determining drug safety, predicting drug-drug interactions, and elaborating appropriate drug administration protocols [43].

2.3 HEPATIC CYP450 AND INFLAMMATION

During inflammatory disorders, proinflammatory cytokines, such as interferon-γ (IFN-γ), interleukin-1 (IL-1), IL-6, and tumor necrosis factor α (TNF-α), are produced and released from monocytes, macrophages, and stromal cells [44]. Proinflammatory cytokines bind to receptors (e.g., Toll-like receptors) on the cell surface of Kupffer cells in the liver. These receptors mediate the inflammatory response, activating intracellular signaling systems and regulating the gene transcription of transporters and enzymes [45]. Thus, impairments in hepatic drug disposition during inflammation have been attributed to downregulation of the gene expression of drug-metabolizing enzymes, especially CYPs and drug transporters [46–48]. The expression or activity of CYP generally decreases during chronic inflammation, during lipopolysaccharide (LPS) exposure, and in the presence of proinflammatory cytokines (e.g., IL-6, TNF-α, and IFN-γ). In this case, changes in CYP expression can alter the pharmacokinetics of prescription medications, increasing plasma levels of substances and resulting in toxicity or side-effects [2, 48].

Primary hepatocytes and animal models have been used to evaluate the effects of cytokines on CYP expression and activity. The response to inflammation depends on the type of disease, disease state, time course, and CYP involvement. Therefore, predicting changes in drug exposure in patients is difficult, demonstrating the importance of developing endogenous markers of CYP activity to individually tailor drug dosages and the choice of therapy [2].

To evaluate the effects of inflammation on CYPs, phase II enzymes, and transporters (i.e., Ugt1a1, Sultn, Mrp2), Shah et al. [46] applied LPS (i.e., Gram-negative bacteria) and lipotechoic acid (LTA; Gram-positive bacteria) to mouse hepatocytes. Lipopolysaccharide and LTA similarly downregulated drug-metabolizing enzymes. Lipopolysaccharide promoted the downregulation of enzymes in the absence of CARs. Lipotechoic acid attenuated the suppressive effect of LPS in the absence of CARs, indicating the involvement of CARs in negative regulation that is mediated by LTA in those genes. The effects on genes depend on the inflammatory stimulus, which can change among Gram-positive and Gram-negative bacteria. This indicates that patients who are exposed to bacterial infections can present differences in drug metabolism [46]. Moriya et al. [11] analyzed the effect of LPS treatment on mRNA levels of CYPs that are regulated by xenobiotic-activated nuclear receptors. They reported a decrease in mRNA levels of CYP3A11, CYP2C29, CYP2C55, and CYP1A2 in LPS-induced inflammatory responses. Considering that these effects were mediated by PXRs and AhRs and possibly CARs in the liver in healthy mice, the authors suggested that this decrease depends on TNF-α and IL-1β that are reduced in the liver in mice that are treated with the PXR/CAR/AhR activator + LPS [11].

The above processes have been extensively studied in animal models but less so in humans, and even less is known about responses in the absence of inducing agents [47]. Aitken and Morgan [47] investigated the effects of LPS, IL-1β, IL-6, TNF-α, IFN-γ, and transforming growth factor β (TGF-β) on the mRNA expression of CYP2B6 and CYP2C in human hepatocytes, comparing with responses of CYP3A4. The authors observed negative regulation for all CYPs when in contact with IL-6. For other cytokines, each CYP presented a specific response. For example, the expression of CYP2C9 and CYP2C19 decreased while in the presence of IL-6 and TGF-β, but expression was unaltered in the presence of LPS, TNF-α, IFN-γ, and IL-1. CYP3A4 and CYP2C8 were downregulated by all cytokine treatments. Others enzymes (e.g., hepatic flavin monooxygenases, UDP-glucuronosyltransferases, sulfotransferases, and glutathione-*S*-transferases) and hepatic transporters also decreased in inflammatory conditions (e.g., hepatitis, cirrhosis, LPS-induced cholestasis, and LPS-induced inflammation) [48].

CYP2 is an important family of enzymes that are involved in inflammatory processes. In mice, CYP2A5 was induced by inflammatory conditions and infectious

disease, which decreased the regulation and activity of several CYP isoforms [49]. CYP2C and CYP2J epoxygenases are responsible for converting arachidonic acid to antiinflammatory epoxyeicosatrienoic acid (EET), which exerts protective effects in various disorders, including cardiovascular disease and metabolic syndrome [2]. The downregulation of CYP epoxygenase and decreases in EET levels in hepatic tissue are a consequence of fatty liver disease-associated inflammation, thus justifying therapeutic approaches that target these pathways [50]. Raffaele et al. [51] reported an increase in IL-10 mRNA level, decreases in TNFα and NF-κB mRNA levels, fatty acid accumulation, fibrosis, and non-alcoholic fatty liver disease (NAFLD) in db/db/ mice (i.e., a model of obesity) that were treated with EET, thus demonstrating that EET may be a pharmacological strategy to prevent fibrosis, mitochondrial dysfunction, and the development of NAFLD [51]. The CYP/EET pathway was also shown to modulate renal dysfunction in advanced cirrhosis in rats that were subjected to bile duct ligation [52].

CYP4A and CYP4F hydroxylases are responsible for metabolizing multiple substrates that are related to regulating inflammation and lipid homeostasis. One challenge, however, is determining which substrates are physiologically relevant for each enzyme. The best-characterized activities include the generation of 20-hydroxyeicosatetraenoic acid (20-HETE) and inactivation of leukotriene B4 [2]. 20-HETE induces endothelial inflammation by increasing the expression of adhesion molecules, cytokines, and chemokines in leukocytes [53].

Considering the features of CYPs, they have been the focus of studies of their involvement in the variability of drug efficacy and toxicity in inflammatory states [2, 54]. Fig. 2 summarizes the influence of inflammation on CYP expression.

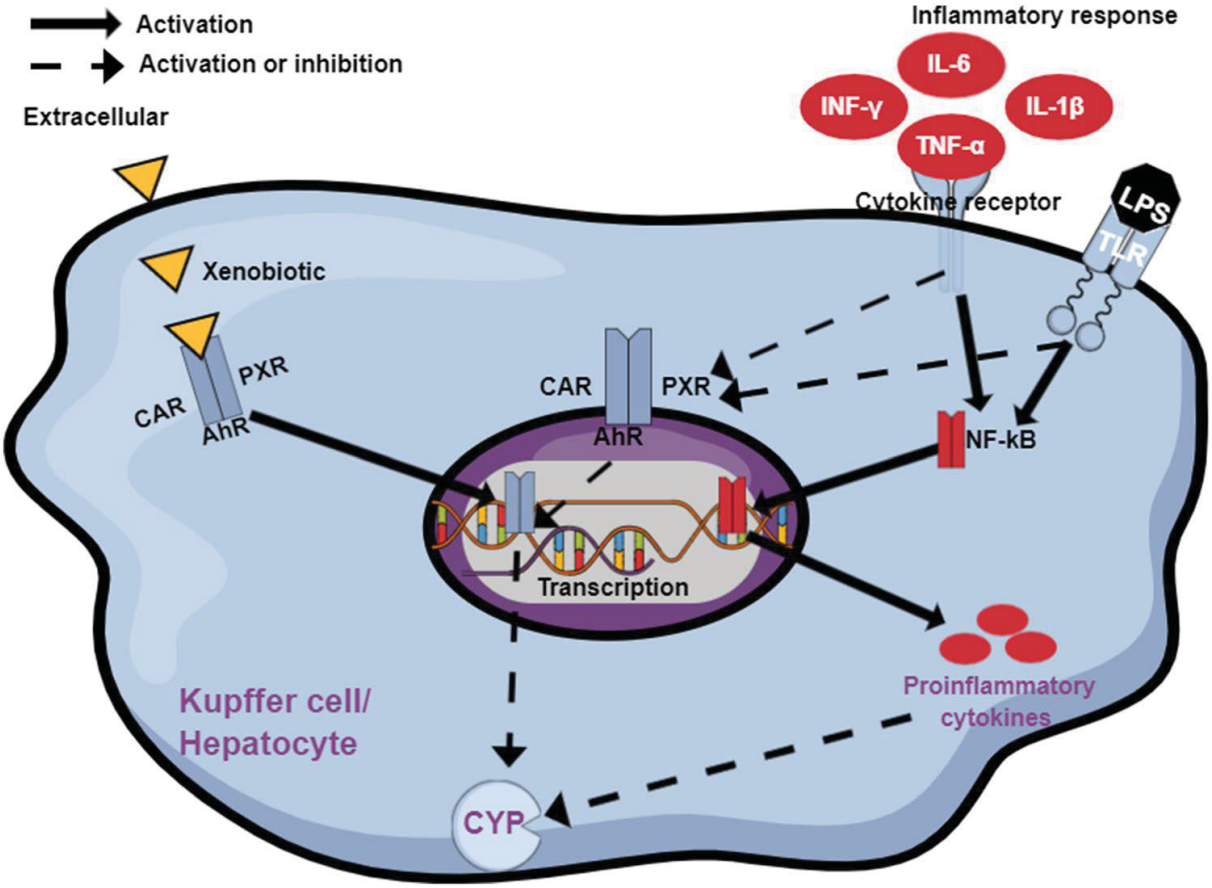

Figure 2 - Regulation of CYP enzyme transcription by inflammatory processes in the liver.
(Figure produced by Mind the Graph software)

2.4 RELATIONSHIP BETWEEN CYP450 AND CANCER

Carcinogen metabolism is complex and mediated by the activity of single or multiple genes, which limits the ability to predict cancer risk that involves CYP modulation [55]. The CYP family is involved in the metabolism of many pre-carcinogens, resulting in secondary metabolites that can cause DNA damage, generate chemical adducts, and act on the activation or inactivation of anticancer drugs [56, 57].

Approximately 1% of the human population presents stable variations of gene sequences or polymorphisms [58]. Polymorphic CYP enzymes play an important role in detoxication via the metabolism of substances, and these may account for interpatient variability in the clinical course of various cancers [59]. Associations between gene polymorphisms and cancer susceptibility can be revealed by CYP1A1, CYP1A2, and CYP2E1, which are responsible for the biotransformation

of chemicals, especially the metabolic activation of pre-carcinogens. Polymorphisms of CYP genes that are involved in drug metabolism, especially CYP2C9, CYP2C19, CYP2D6, CYP3A4, and CYP3A5, can result in therapeutic failure or side effects [55].

Agundez [60] performed phenotyping analyses and found an association between CYP enzyme activity and the risk of developing several forms of cancer, such as lung, liver, head, and neck cancers. Controversial findings suggest that colorectal and prostate cancers may be associated with CYP polymorphisms, but no evidence of such association with bladder cancers has been reported. Several polymorphisms of CYP enzymes occur because of gene duplication, gene deletion, or single-nucleotide polymorphisms. Generally, the enzymes that have been the most extensively studied with regard to tumors are CYP1A1, CYP1A2, CYP1B1, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C18, CYP2C19, CYP2D6, CYP2E1, CYP3A4, and CYP3A5 [60].

The expression of downstream genes of the CYP1 family (e.g., CYP1A1, CYP1A2, and CYP1B1) is initiated by AhRs, a ligand-activated transcriptional factor that dimerizes with aryl hydrocarbon receptor nuclear translocator (ARNT), resulting in a complex that binds to xenobiotic response elements (XREs). The CYP1 family is involved in the metabolism of endogenous hormones, xenobiotics, and drugs. The expression of CYP1 can be regulated by estradiol (E2) or xenobiotics in diverse cancers [61]. For example, CYP1B1 is responsible for converting E2 into 4-hydroxyestradiol, which can then be converted to potential carcinogens by peroxidases, forming estradiol-3,4-quinone. CYP1B1 is overexpressed in tumors. It has been shown to be active within tumors and capable of metabolizing many anticancer drugs [62]. High expression of the CYP1 family indicates the possibility of carcinogenesis through xenobiotic exposure in endometrial and ovarian cancers [61].

The CYP11 family is important in steroidal biosynthesis. CYP11B1 and CYP11B2 exhibit the highest number of mutations, and ~80% of these mutations can affect the conformation of proteins and subsequently affects their function. Lower CYP11 expression and CYP11 mutations influence steroidal biosynthesis [8]. CYP11A1 expression presented a negative regulatory effect on colon adenocarcinoma, renal clear cell carcinoma, liver hepatocellular carcinoma (HCC), lung squamous cell carcinoma, prostate adenocarcinoma, and uterine corpus endometrial carcinoma.

The human CYP4 family is also important in cancer development. CYP4A11, CYP4F2, and CYP4F3B hydroxylate arachidonic acid at the omega position to form hydroxyeicosatetranoic acid (20-HETE), which has important effects on tumor progression, angiogenesis, and blood pressure regulation in the vasculature and kidneys [63, 64]. Table 2 summarizes the main CYPs that are related to cancer [60]. CYP polymorphisms are not solely associated with pro-tumoral activity. In a meta-analysis of hormone-related cancer risk, the polymorphic *CYP24A1 rs2296241* single-nucleotide polymorphism was associated with a lower risk of prostate cancer [65]. CYPs also influence HCC, breast cancer, and lung cancer.

Table 2 - Main CYP isoforms that are involved in the carcinogenesis of different types of cancer.

CYP	Cancer
CYP1A1	Lung cancer, head cancer, neck cancer, oral cancer, larynx/pharynx cancer, esophageal cancer, colorectal cancer, gallbladder cancer, breast cancer, prostate cancer, renal cancer, endometrioid and ovarian cancer, lymphomas, leukemia
CYP1A2	Urothelial and bladder cancer
CYP1B1	Prostate cancer, ovarian cancer, head cancer, neck cancer, breast cancer, lung cancer, gastrointestinal cancer, bladder cancer, liver cancer
CYP2A6	Lung cancer, oral cancer, colorectal cancer
CYP2C9	Colorectal cancer, lung cancer
CYP2C18 and CYP2C19	Prostate cancer, bladder cancer, lung cancer, liver cancer, colorectal cancer, acute leukemia
CYP2D6	Lung cancer, head cancer, neck cancer, liver cancer, melanoma, breast cancer, acute leukemia, prostate cancer, bladder cancer, brain cancer, renal cancer, colorectal cancer, ovarian cancer, anal cancer, vulvar cancer, pancreatic cancer, cervix cancer, pituitary cancer, lymphomas
CYP2E1	Lung cancer, digestive tract cancer
CYP3A4	Prostate cancer, breast cancer, ovarian cancer, myeloid leukemia
CYP3A5	Acute myeloid leukemia, myelodysplastic syndrome, acute lymphoblastic leukemia

Source: [60].

2.4.1 CYP and hepatocellular carcinoma

Liver cancer is the sixth most common malignancy and fourth leading cause of cancer-associated mortality [66, 67], including hepatocellular carcinoma that comprises 75-85% of cases. In most high-risk HCC areas (e.g., China and Eastern

Africa), the key determinants are chronic HBV infection and aflatoxin exposure. In other countries (e.g., Japan and Egypt), HCV infection is likely the predominant cause [67]. HCC is accompanied by liver dysfunction. The activity of CYP isoforms is differentially affected in HCC patients, and personalized treatments are recommended [68, 69]. CYP genes are involved in the pathogenesis of HCC [70]. A study of liver tissues from HCC patients reported an increase in the intrinsic clearance of CYP2C9, CYP2D6, and CYP2E1 and a decrease in the intrinsic clearance of CYP1A2, CYP2C8, and CYP2C19 compared with non-tumor liver tissues (controls) that were obtained from patients with liver hemangioma, metastatic carcinoma, cholelithiasis, and gallbladder cancer. CYP2D6 exhibited an increase in activity (Km parameter) in HCC patients with comorbid cirrhosis. Genetic polymorphisms, such as *CYP2D6*10*, *CYP2C9*3*, and *CYP3A5*3*, can affect enzymatic function and predict HCC risk [69].

Gao et al. [68] evaluated 102 HCC patients and identified different clearance rates. Based on the clearance of CYP isoform-specific substrates at the microsomal level, CYP2C9, CYP2D6, and CYP2E1 were significantly increased in HCC patients, whereas the values for CYP1A2, CYP2C8, and CYP2C19 decreased. CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C19, CYP3A4, and CYP3A5 levels, indicated by microsomal protein per gram of liver, were significantly reduced in HCC patients. The authors suggested that such diseases as fibrosis and cirrhosis and polymorphisms of CYP genes influence the hepatic clearance of CYP [68].

Another important CYP in this context is CYP2J2, an arachidonic acid epoxygenase that is related to hyperhomocysteinemia (HHcy). This condition is characterized by an increase in homocysteine (Hcy) [71], which can be associated with the rapid proliferation of tumor cells, including HCC cells [72, 73]. Hyperhomocysteinemia promotes hepatocarcinogenesis via metabolism and CYP2J2 signaling. Elevations of Hcy can result from liver disease or dysfunction and alter intracellular lipid metabolism. CYP2J2 is also expressed in other cancers (e.g., breast cancer, melanoma, and leukemia) and promotes metastasis [71]. Zhang et al. [71] reported higher levels of 4-epoxyeicosatrienoic (EET) isomers, CYP2J2, and intracellular Hcy in 42 HCC cases compared with non-tumor tissue. Increases in Hcy levels favor the neoplastic cellular phenotype, which is reversed by the absence of CYP2J2.

Another concern is when HCC is associated with viral infection. Yan et al. [74] evaluated the protein and mRNA levels of CYP1A2, CYP2A6, CYP2C8, CYP2C9, CYP2D6, CYP2E1, and CYP3A4 in tumors and pericarcinomatous tissues from patients with hepatitis B virus-positive HCC. The authors found 2.5- to 30-fold lower maximal metabolic velocity (V_{max}) of tumor microsomes enzymes and decreases in their protein and mRNA expression levels, demonstrating serious impairments in CYP activity [74]. Another study analyzed HCC that arose from hepatitis C virus (HCV)-infected livers. The levels of CYP2B6, CYP2C9, CYP2C19, CYP3A5, CYP4F3, and CYP27A1 were significantly lower and the levels of CYP2E1 and CYP4F2 were slightly lower in HCC with venous invasion than in HCC without venous invasion [70]. Thus, virus invasion decreases the levels of important CYPs, suggesting that CYPs may be therapeutic targets for virus-associated HCC [70, 74].

Considering the enormous diversity of CYPs and their high concentrations and activity in the liver, the ability to predict the effects of HCC on drug clearance may be helpful for designing clinical studies and clinically managing pharmacotherapy in HCC patients [68]. The activity of CYPs from families 1, 2, and 3 is altered in HCC, mainly CYP2 subfamilies (A, B, C, D, E, and J).

2.4.2 CYP and breast cancer

In breast cancer, CYP metabolizes endogenous substrates (e.g., estradiol) and exogenous substrates (e.g., anticancer drugs). These enzymes are responsible for generating DNA-damaging pro-carcinogens and the response to antiestrogen therapies [75, 76].

CYP3A is also involved in the metabolism of tamoxifen, an important therapeutic agent in estradiol receptor-positive breast cancer. Variations of CYP3A expression in breast cancer tissues among ethnic groups might result in differences in therapeutic efficacy. Oyama et al. [77] immunohistochemically detected the absence of expression of this enzyme in cancer specimens from 34 Japanese patients. The expression of CYP3A may be less frequent in the Japanese population, whereas its expression has been reported in 20% of breast cancer cases in Caucasians.

Floriano-Sanchez et al. [78] found a significant increase in CYP3A4 expression in breast cancer stroma and gland regions compared with healthy tissue. Various factors, such as smoking, alcoholism, and hormonal contraceptive use, are

significantly associated with CYP3A4 protein expression. The authors suggested that CYP3A4 expression promotes breast cancer development and can be used as a predictor of the tumor response to different treatments. Vaclavikova et al. [79] found negligible levels of CYP3A4 and CYP2C9 expression, whereas CYP1B1 expression was ~50-fold higher than CYP2E1 expression, and this overexpression was detected in one-third of tumors. Higher CYP2E1 expression was associated with an invasive lobular type of tumor, locally advanced disease as well as with non-tumor tissue of progesterone receptor-negative patients, indicating that CYP2E1 may be a prognostic marker in breast cancer [79]. A positive association was also found between CYP1B1 expression in peripheral blood lymphocytes and non-tumor tissues. The correlation between CYP1B1 expression in breast tumor samples and peripheral blood lymphocytes was weaker. The expression of CYP1B1 in tumor cells may influence the metabolism or activation of environmental carcinogens or the drug response [79].

Estrogen receptors (ERs) are expressed in breast cancers. E2 was shown to increase the level of CYP1B1, which was reversed by the estrogen receptor antagonists ICI 182,780 and 4-hydroxytamoxifen, indicating that expression of the CYP1 family in regions downstream from AhRs is regulated by the activation of ERs [61].

Parada et al. [80] found a correlation between single-nucleotide polymorphisms of CYP genes and the incidence of breast cancer, and interactions were found with the consumption of smoked/grilled meat, which is an important source of polycyclic aromatic hydrocarbons. However, one example of a CYP polymorphism that is not responsible for cancer development is CYP1A1, which is expressed in extra-hepatic tissues (e.g., the breast) and responsible for polycyclic aromatic hydrocarbon metabolism and estrogen metabolism. CYP1A1 can present four polymorphisms: *3801T/C*, *Ile462Val*, *3205T/C*, and *Thr461Asp*. Although there are ethnic differences in the presence of these polymorphisms, none are related to breast cancer [81]. The highly polymorphic enzyme CYP2D6 can influence the success of breast cancer therapy (see section 5 below). CYPs are associated with both breast tumor development and progression and the efficacy of cancer treatment [77].

2.4.3 CYP and lung cancer

The pathogenesis of lung cancer is strongly associated with environmental chemical exposure, particularly tobacco smoke and asbestos. Many compounds require enzymatic activation by CYPs to exert their deleterious effects on pulmonary cells. Differences in carcinogenesis are mainly attributable to interindividual differences in the capacity to activate or inactivate chemical toxicants in cellular pathways that are involved in CYP regulation and lung carcinogenesis [82–84].

Various CYPs, such as CYP1A1 (in smokers), CYP1B1, CYP2B6, CYP2E1, CYP2J2, and CYP3A5, are mostly expressed in bronchial and bronchiolar epithelia, Clara cells, type II pneumocytes, and alveolar macrophages in human lung tissues [83]. Determining the local expression of CYP enzymes in lung cancer and surrounding tissues is an important determinant of anticancer drug efficacy and the development of individualized treatment [84].

Oyama et al. [4] mentioned the expression of AhRs, CYP1A1, CYP2A6, CYP2E1, and CYP3A in lung adenocarcinoma but not in squamous cell cancers. This expression in adenocarcinoma was more frequent in females than in males, suggesting that the expression of these enzymes and receptors may be associated with a higher risk of developing lung adenocarcinoma in women [4]. CYP19 (aromatase) converts testosterone to estrogen and was found in more than 80% of non-small-cell lung tumors. CYP24A1, which converts 1α,25-dihydroxyvitamin D3 to its inactive 24-hydroxylated derivatives, was detected in lung cancer [84]. Moreover, the inactivation of 1,25-OH D3 by CYP24A1 in tumor tissues is associated with a poor prognosis of some human cancers [4].

Song et al. [85] showed that CYP1A1 plays an important role in the activation of tobacco carcinogens. Polymorphisms of CYP1A1 are important predictors of the susceptibility to lung cancer, which has received particular research interest in recent years. Several polymorphisms of the CYP1A1 locus have been identified, the frequencies of which in the population depend on ethnicity. In the Chinese population, for example, the *CYP1A1m1* and *CYP1A1m2* polymorphisms are associated with smoking-related lung cancer risk [85].

Lung cancers have features that make these tumors different from others because they are influenced by tobacco, gender, and the metabolism of steroid hormones and vitamin D, thus involving both xenobiotic metabolizing CYPs and steroidogenic CYPs. The spectrum of CYP expression in lung tumors may be useful

markers for tumor classification and diagnosis and the management of lung cancer therapy [4].

2.5 CYP IN CHEMOTHERAPY: FUNCTIONS AND INTERFERENCE

As previously mentioned, the CYP family is involved in the metabolism of various anticancer drugs. Consequently, variations of CYP enzymes (e.g., polymorphisms, induction, and inhibition) can interfere with anticancer drug effects [86], especially because classic chemotherapy is not necessarily specific to tumor cells and also acts on healthy cells [87].

Mutations of CYP enzymes can be divided into four phenotypes: poor metabolizers (two nonfunctional genes), intermediate metabolizers (one allele deficiency), extensive metabolizers (two copies of normal genes), and ultrarapid metabolizers (three or more functional active gene copies) [88]. These phenotypes can result in therapeutic failure or cause side effects in patients during chemotherapy. The main side effects of the doxorubicin and cyclophosphamide (AC) protocol is neutropenia. Polymorphisms of CYP2B6 and excision endonuclease and repair cross-complementary 1 (ERCC1) were shown to be related to the exacerbation of stage 4 neutropenia in patients who received the AC protocol. These polymorphisms may be strong independent predictors of stage 4 neutropenia, in addition to the initial total count of white blood cells and body mass index [89].

Ahmed et al. [90] reported that cisplatin and paclitaxel inhibited CYP2E1 and CYP3A1/2 in isolated hepatic microsomes in rats. Paclitaxel dose-dependently inhibits CYP3A. One study found that turmeric pretreatment enhanced the inhibitory effect of cisplatin and paclitaxel on CYP3A1/2 in isolated rat hepatic microsomes, with a reduction of their inhibitory constants (K_i). The inhibitory effects of these chemotherapies on CYP2E1 were attenuated by turmeric pretreatment. Kostrubsky et al. [91] reported that paclitaxel induced CYP3A4 protein and mRNA expression. These authors also found that paclitaxel and rifampicin had similar enzymatic activity, but higher concentrations of paclitaxel decreased CYP3A activity and immunoreactive protein [92].

Importantly, cisplatin dose-dependently induces renal tubular toxicity. This effect occurs because cisplatin is a potent inducer of CYP4A11 and exerts toxic effects through the induction of CYP4A11 and 20-HETE generation [93]. Cyclophosphamide and ifosfamide are activated by CYP2B and CYP3A,

respectively, but CYP2A6, CYP2B6, CYP2C8, CYP2C9, and CYP3A4 can also be activated by these drugs [94]. Some cancer patients require antidepressant drug treatment, such as with the selective serotonin reuptake inhibitors paroxetine and fluoxetine. These antidepressants have been shown to inhibit the metabolism of tamoxifen by CYP2D6, which may reduce its efficacy [95].

The production of EET by CYP in breast cancer is correlated with cancer progression. Phuong et al. [96] suggested that the CYP3A4-mediated EET pathway may be a therapeutic target for the treatment of tamoxifen-resistant breast cancer [96]. Phytoestrogens and dexamethasone can be regulated by CYP1 family expression, which can compromise cancer therapy [61]. For example, CYP1B1 overexpression was shown to lead to resistance to docetaxel [97], and this effect was reversed by a CYP1B1 inhibitor [98]. Additionally, CYP1B1 promoted the resistance to other chemotherapeutic agents, including tamoxifen [99] and cisplatin [100].

Tamoxifen is an endocrine treatment that is used in pre- and post-menopausal women in whom aromatase inhibitors are contraindicated [95]. Tamoxifen is a pro-drug that is metabolized by CYP2D6, CYP3A4, CYP2B6, and CYP2C19. However, CYP2D6 is a highly polymorphic gene that can lead to null or low enzyme activity, resulting in therapeutic failure. For example, the *CYP2D6*6* allele influences survival in breast cancer patients, whereas the *CYP2D6*4* allele does not. Therefore, some authors have discouraged the genotyping of CYP2D6 [101]. However, Blancas et al. [102] classified the CYP2D6 genotype as slow metabolizers or rapid metabolizers. Patients who were classified as slow CYP2D6 metabolizers had shorter disease-free survival during tamoxifen treatment, but no differences in overall survival (OS) were observed. The authors suggested that no difference in OS was observed because tamoxifen treatment was interrupted in most cases during disease relapse [102]. In the Chinese Han population, *CYP2D6*10* T/T genotype patients exhibited less benefit from tamoxifen treatment [103]. Thus, genotyping CYP2D6 in breast cancer patients is important for individualized tamoxifen therapy [95], but further investigations are necessary to explain how tamoxifen treatment is affected by SNPs of CYP2D6. This confirms the clinical validity of employing pharmaconegomics for tamoxifen therapy [104]. Other common enzyme variants, such as CYP2C19, CYP2C9, and CYP2B6, are also involved in tamoxifen metabolism, but do not present a relation between release-free time in patients with breast cancer [105].

One study [106] demonstrated a relationship between patients with multiple myeloma and the time of administration of cyclophosphamide, interferon, and betamethasone. The authors found that a longer half-life and higher maximal plasma concentrations were associated with a decrease in the clearance of cyclophosphamide when IFN-α was administered previously compared with IFN-α administration 24 h after cyclophosphamide treatment. A cytotoxic metabolite of cyclophosphamide, 4-hydroxy-cyclophosphamide (4-OHCP), is formed through the enzymatic activity of CYP3A4 and affected by INF-α pretreatment relative to cyclophosphamide administration. When IFN-α was administered after cyclophosphamide, a better therapeutic effect was observed [107]. Treatment with high-dose IFN-α2b reduced the activity of CYP1A2 and CYP2C19 by 60% and 40%, respectively, in 17 patients with melanoma [107]. Thus, the IFN-α administration schedule should be considered to achieve better efficacy of alkylating agents that are used for the treatment of multiple myeloma and related diseases [106].

Vincristine metabolism is significantly higher with CYP3A5 than with CYP3A4. In children with precursor B-cell acute lymphoblastic leukemia, CYP3A5 expressers experienced less vincristine-induced peripheral neuropathy and produced more primary metabolites compared with CYP3A5 non-expressers [108]. CYP3A5 genotype is an important determinant of vincristine clearance and exposure in patients. CYP3A5 genotype was suggested to be a strong predictor of vincristine toxicity and may provide a starting point for better dosing strategies for a drug that is critical for the treatment of multiple curable childhood cancers [108]. Moreover, several chemotherapies can interact with CYPs. Table 3 presents enzymes that are responsible for the metabolism of different chemotherapeutic drugs.

Table 3 - CYPs involved in the metabolism of some chemotherapeutic agents.

Drug	CYP involved in metabolism	Metabolism pathway
Cyclophosphamide	CYP2A6, CYP2B6, CYP2C19, CYP2C9, CYP3A4, CYP3A5 [109]	Cyclophosphamide is converted to 4-hydroxycyclophosphamide by CYP2B6, CYP2C9, CYP2C19, CYP3A4, and CYP3A5 or to 2-dechcloroethylcyclophosphamide + chloracetaldehyde by CYP3A4/CYP3A5 [109]
Docetaxel	CYP3A4 [110]	Docetaxel is hydroxylated to hydroxyldocetaxel [111]

Etoposide	CYP3A4 [112]	Etoposide is metabolized to etoposidecatechol
Flutamide	CYP1A2 [113, 114]	Flutamide is metabolized by CYP1A2 to 2-hydroxyflutamide [113, 114]
Ifosfamide	CYP2A6, CYP3A4, CYP3A5, CYP2B6, CYP2C8, CYP2C9, CYP2C19 [114, 115]	The prodrug ifosfamide is activated to 4-hydroxyifosfamide by CYP2B6 and CYP3A4/CYP3A5, whereas CYP2A6, CYP2C8, CYP2C9, and CYP2C19 contribute less to this reaction. CYP2B6 and CYP3A4 convert 4-hydroxyifosfamide to 2-dichloroethyl ifosfamide, 3-dichloroethylifosfamide, and chloroacetaldehyde [114, 115]
Irinotecan	CYP3A4, CYP3A5 [114]	Irinotecan is metabolized by CYP3A4 and CYP3A5 to 7-ethyl-10-[4-*N*-(5-aminopentanoic acid)-1-piperidino] carbonyloxycamptothecin or 7-ethyl-10-[4-(1-piperidino)-1-amino] carbonyloxycamptothecin [114]
Imatinib	CYP3A4, CYP3A5 [114, 116]	Imatinib is demethylated by CYP3A4 and CYP3A5 to N-demethyl-imatinib; CYP1A2, CYP2C8, CYP2C9, CYP2C19, and CYP2D6 can hydroxylate this drug [114, 116]
Paclitaxel	CYP2C8, CYP3A4 [117, 118]	Paclitaxel is converted to 6-α-hydroxypaclitaxel by CYP2C8, which is converted to dihydroxypaclitaxel by CYP3A4 and to 3'-p-hydroxypaclitaxel by CYP3A4, which is converted to dihydrodypaclitaxel by CYP2C8 [114]
Sorafenib	CYP3A4 [119]	Sorafenib is metabolized by CYP3A4 to pyridine N-oxide (M2) [119]
Tamoxifen	CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP3A4, CYP3A5 [101, 109]	CYP2D6, CYP2B6, CYP2C19, CYP3A4, CYP3A5, and CYP2D9 oxidize tamoxifen to 4-hydroxytamoxifen; CYP3A4 and CYP3A5 convert tamoxifen to N-desmethyltamoxifen and after to N-didesmethyltamoxifen; N-desmethyltamoxifen is converted to endoxifen by CYP2D6, and 4-hydroxytamoxifen is converted to endoxifen by CYP3A4 and CYP3A5 [101]
Tegafur	CYP2A6 [120]	Tegafur [*R*,*S*-1-1(tetrahydrofuran-2-yl)-5-FU] is a prodrug that is converted to 5'-hydroxytegafur by CYP2A6 and then to 5-fluorouracil [120]
Vincristine and vinca alkaloids (vinblastine, vindesine)	CYP3A4, CYP3A5 [108]	

Note: Tegafur, also called ftorafur, is a constituent of UFT, an oral fluoropyrimidine, designed in 1978 by adding uracil to ftorafur [121] and S-1 that consists of three pharmacological agents (at a molar ratio of 1:0.4:1), namely tegafur (FT), 5-chloro-2-4-dihydroxypyridine (CDHP) and oxonic acid (Oxo) [122].

2.6 CONCLUSIONS

CYP enzymes are important for the biotransformation of xenobiotics and are also related to cancer development and the inflammation. The inflammatory process is usually present in the tumor microenvironment and systemically. Cytokines (e.g., IL-1, IL-6, and TNF-α) that are released can then decrease the expression of CYP genes, especially from the CYP1, CYP2, CYP3, and CYP4 families (Fig. 3). Alterations of CYPs in cancer may influence the metabolism of chemotherapeutic drugs, in which CYP enzymes may be inhibited in cancer patients, especially in polychemotherapy protocols. Polymorphisms of CYP enzymes or their hyperactivity can result in gene mutations, cancer development, or the inactivation of chemotherapeutic drugs. Studies of CYPs that are influenced by the inflammatory process are necessary for predicting drug-drug interactions, including chemotherapies and other medications that are prescribed for cancer patients (e.g., antidepressants and antiemetics), and establishing efficient therapies with fewer side effects.

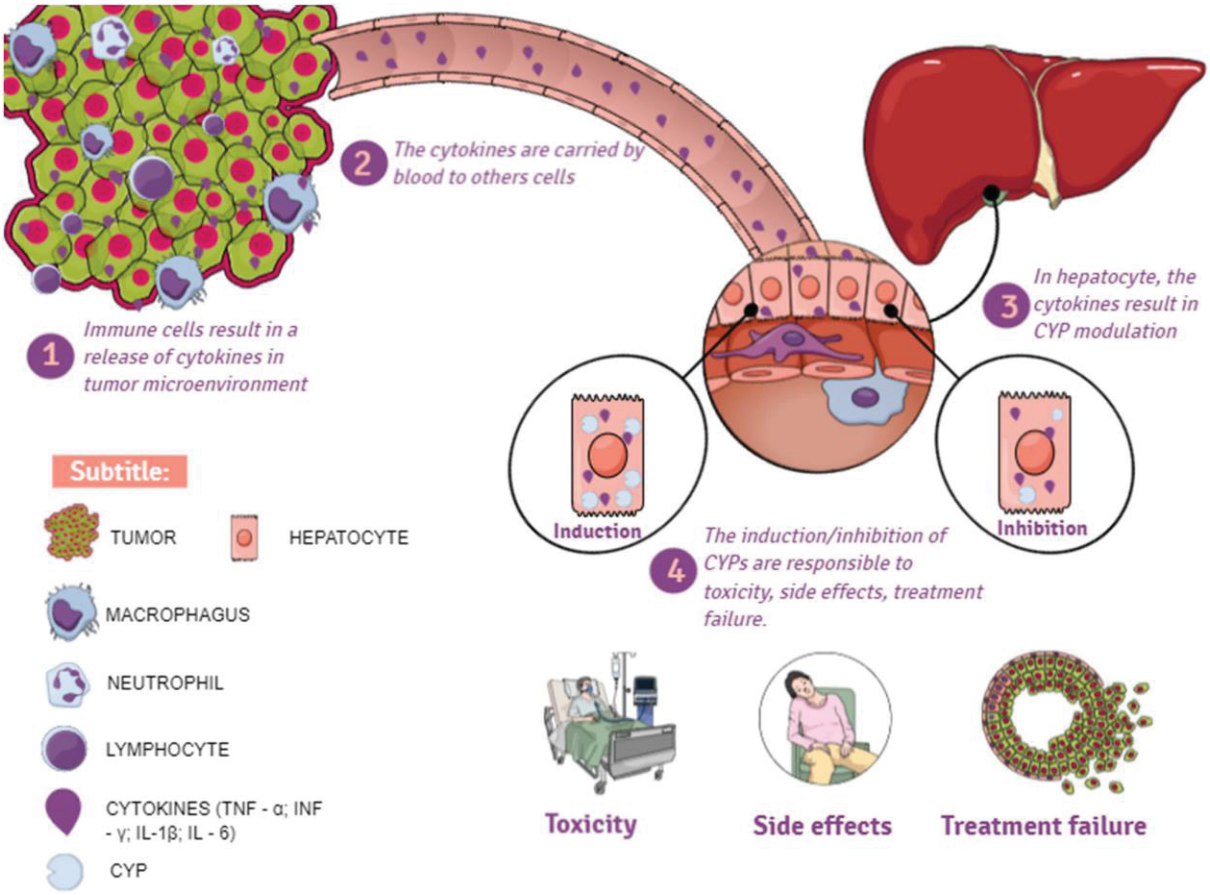

Figure 3 - Relationship between CYP and inflammation in cancer patients.
(Figure produced by Mind the Graph software)

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2.9 CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest

2.10 AVAILABILITY OF DATA AND MATERIAL

Not applicable.

2.11 CODE AVAILABILITY

Not applicable.

2.12 AUTHORS CONTRIBUTION

MCS was responsible for conceptualization, literature review and writing; AA was responsible for the manuscript format, writing and editing.

2.13 REFERENCES

1. Stavropoulou E, Pircalabioru GG, Bezirtzoglou E (2018) The Role of Cytochromes P450 in Infection. Front Immunol 9:1–7. https://doi.org/10.3389/fimmu.2018.00089

2. Christmas P (2015) Role of Cytochrome P450s in Inflammation. In: Advances in Pharmacology, 1st ed. Elsevier Inc., pp 163–192

3. He X, Feng S (2015) Role of Metabolic Enzymes P450 (CYP) on Activating Procarcinogen and their Polymorphisms on the Risk of Cancers. Curr Drug Metab 16:850–863. https://doi.org/10.2174/1389200216101512101645O1

4. Oyama T (2007) Cytochrome P450 expression (CYP) in non-small cell lung cancer. Front Biosci 12:2299. https://doi.org/10.2741/2232

5. Korobkova EA (2015) Effect of Natural Polyphenols on CYP Metabolism: Implications for Diseases. Chem Res Toxicol 28:1359–1390. https://doi.org/10.1021/acs.chemrestox.5b00121

6. Guengerich F (2014) Analysis and Characterization of Enzymes and Nucleic Acids Relevant to Toxicology. In: Hayes' Principles and Methods of Toxicology, Sixth Edition. CRC Press, pp 1905–1964

7. Nebert DW, Wikvall K, Miller WL (2013) Human cytochromes P450 in health and disease. Philos Trans R Soc Lond B Biol Sci 368:20120431. https://doi.org/10.1098/rstb.2012.0431

8. Fan Z, Wang Z, Chen W, et al (2016) Association between the CYP11 family and six cancer types. Oncol Lett 12:35–40. https://doi.org/10.3892/ol.2016.4567

9. Furge LL, Guengerich FP (2006) Cytochrome P450 enzymes in drug metabolism and chemical toxicology: An introduction. Biochem. Mol. Biol. Educ. 34:66–74

10. Omura T, Sato R (1964) The Carbon Monoxide-Binding Pigment of Liver Microsomes. Ii. J Biol Chem 239:2379–2385

11. Moriya N, Kataoka H, Fujino H, et al (2012) Effect of lipopolysaccharide on the xenobiotic-induced expression and activity of hepatic cytochrome P450 in mice. Biol Pharm Bull 35:473–80. https://doi.org/10.1248/bpb.35.473

12. Van der Weide J, Steijns LS (1999) Cytochrome P450 enzyme system: genetic polymorphisms and impact on clinical pharmacology. Ann Clin Biochem 36 (Pt 6):722–729

13. Zanger UM, Schwab M (2013) Cytochrome P450 enzymes in drug metabolism: Regulation of gene expression, enzyme activities, and impact of genetic variation. Pharmacol Ther 138:103–141. https://doi.org/10.1016/j.pharmthera.2012.12.007

14. Robottom-Ferreira AB, Aquino SR, Queiroga R, et al (2003) Expression of CYP2A3 mRNA and its regulation by 3-methylcholanthrene, pyrazole, and ??-ionone in rat tissues. Brazilian J Med Biol Res 36:839–844. https://doi.org/10.1590/S0100-879X2003000700003

15. Gaudet P, Livstone MS, Lewis SE, Thomas PD (2011) Phylogenetic-based propagation of functional annotations within the Gene Ontology consortium. Brief Bioinform 12:449–462. https://doi.org/10.1093/bib/bbr042

16. Jeong S, Nguyen PD, Desta Z (2009) Comprehensive in vitro analysis of voriconazole inhibition of eight cytochrome P450 (CYP) enzymes: Major effect on CYPs 2B6, 2C9, 2C19, and 3A. Antimicrob Agents Chemother 53:541–551. https://doi.org/10.1128/AAC.01123-08

17. Gopalakrishnan R, Gupta A, Carlton PS, et al (2002) Functional role of cytochrome p-450 2a3 in N-nitrosomethylbenzylamine metabolism in rat esophagus. J Toxicol Environ Health A 65:1077–1091. https://doi.org/10.1080/152873902760125237

18. Yano JK, Hsu M-H, Griffin KJ, et al (2005) Structures of human microsomal cytochrome P450 2A6 complexed with coumarin and methoxsalen. Nat Struct Mol Biol 12:822–3. https://doi.org/10.1038/nsmb971

19. Tanner J-A, Tyndale R (2017) Variation in CYP2A6 Activity and Personalized Medicine. J Pers Med 7:18. https://doi.org/10.3390/jpm7040018

20. Kilanowicz A, Czekaj P, Sapota A, et al (2015) Developmental toxicity of hexachloronaphthalene in Wistar rats. A role of CYP1A1 expression. Reprod Toxicol 58:93–103. https://doi.org/10.1016/j.reprotox.2015.09.005

21. Lee AJ, Conney AH, Zhu BT (2003) Human cytochrome P450 3A7 has a distinct high catalytic activity for the 16??-hydroxylation of estrone but not 17??-estradiol. Cancer Res 63:6532–6536

22. Laine JE, Auriola S, Pasanen M, Juvonen RO (2009) Acetaminophen bioactivation by human cytochrome P450 enzymes and animal microsomes. Xenobiotica 39:11–21. https://doi.org/10.1080/00498250802512830

23. Kawajiri K, Nakachi K, Imai K, et al (1990) Identification of genetically high risk individuals to lung cancer by DNA polymorphisms of the cytochrome P450IA1 gene. FEBS Lett 263:131–3. https://doi.org/10.1016/0014-5793(90)80721-T

24. Gupta RP, He YA, Patrick KS, et al (2005) CYP3A4 is a vitamin D-24- and 25-hydroxylase: Analysis of structure function by site-directed mutagenesis. J Clin Endocrinol Metab 90:1210–1219. https://doi.org/10.1210/jc.2004-0966

25. Anwar-Mohamed A, Elbekai RH, El-Kadi AOS (2009) Regulation of CYP1A1 by heavy metals and consequences for drug metabolism. Expert Opin Drug Metab Toxicol 5:501–521. https://doi.org/10.1517/17425250902918302

26. Sciarra A, Pintea B, Nahm JH, et al (2017) CYP1A2 is a predictor of HCC recurrence in HCV-related chronic liver disease: A retrospective multicentric validation study. Dig Liver Dis 49:434–439. https://doi.org/10.1016/j.dld.2016.12.002

27. Kot M, Daniel WA (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. https://doi.org/10.1016/j.bcp.2008.05.025

28. Butler MA, Iwasaki M, Guengerich FP, Kadlubar FF (1989) Human cytochrome P-450PA (P-450IA2), the phenacetin O-deethylase, is primarily responsible for the hepatic 3-demethylation of caffeine and N-oxidation of carcinogenic arylamines. Proc Natl Acad Sci U S A 86:7696–7700. https://doi.org/10.1073/pnas.86.20.7696

29. Shimada T, Mernaugh RL, Guengerich FP (2005) Interactions of mammalian cytochrome P450, NADPH-cytochrome P450 reductase, and cytochrome b5enzymes. Arch Biochem Biophys 435:207–216. https://doi.org/10.1016/j.abb.2004.12.008

30. Sansen S, Yano JK, Reynald RL, et al (2007) Adaptations for the oxidation of polycyclic aromatic hydrocarbons exhibited by the structure of human P450 1A2. J Biol Chem 282:14348–14355. https://doi.org/10.1074/jbc.M611692200

31. Lynch T, Price A (2007) The effect of cytochrome P450 metabolism on drug response, interactions, and adverse effects. Am Fam Physician 76:391–6

32. Qian L, Zolfaghari R, Ross a C (2010) Liver-specific cytochrome P450 CYP2C22 is a direct target of retinoic acid and a retinoic acid-metabolizing enzyme in rat liver. J Lipid Res 51:1781–92. https://doi.org/10.1194/jlr.M002840

33. Waxman DJ, Attisano C, Guengerich FP, Lapenson DP (1988) Human liver microsomal steroid metabolism: Identification of the major microsomal steroid hormone 6β-hydroxylase cytochrome P-450 enzyme. Arch Biochem Biophys 263:424–436. https://doi.org/10.1016/0003-9861(88)90655-8

34. Fisher CDC, Lickteig AJA, Augustine LML, et al (2009) Hepatic cytochrome P450 enzyme alterations in humans with progressive stages of nonalcoholic fatty liver disease. Drug Metab Dispos 37:2087–2094. https://doi.org/10.1124/dmd.109.027466.

35. Subramanian M, Low M, Locuson CW, Tracy TS (2009) CYP2D6-CYP2C9 Protein-Protein Interactions and Isoform-Selective Effects on Substrate Binding and Catalysis. Drug Metab Dispos 37:1682–1689. https://doi.org/10.1124/dmd.109.026500

36. Fagerberg L, Hallström BM, Oksvold P, et al (2014) Analysis of the Human Tissue-specific Expression by Genome-wide Integration of Transcriptomics and Antibody-based Proteomics. Mol Cell Proteomics 13:397–406. https://doi.org/10.1074/mcp.M113.035600

37. Williams PA, Cosme J, Vinkovic DM, et al (2004) Crystal structures of human cytochrome P450 3A4 bound to metyrapone and progesterone. Science 305:683–686. https://doi.org/10.1126/science.1099736

38. Ward BA, Morocho A, Kandil A, et al (2004) Characterization of human cytochrome P450 enzymes catalyzing domperidone N-dealkylation and hydroxylation in vitro. Br J Clin Pharmacol 58:277–287. https://doi.org/10.1111/j.1365-2125.2004.02156.x

39. Molowa DT, Schuetz EG, Wrighton SA, et al (1986) Complete cDNA sequence of a cytochrome P-450 inducible by glucocorticoids in human liver. Proc Natl Acad Sci USA 83:5311–5315. https://doi.org/10.1073/pnas.83.14.5311

40. Korhonen T, Turpeinen M, Tolonen A, et al (2008) Identification of the human cytochrome P450 enzymes involved in the in vitro biotransformation of lynestrenol and norethindrone. J Steroid Biochem Mol Biol 110:56–66. https://doi.org/10.1016/j.jsbmb.2007.09.025

41. Waxman DJ, Attisano C, Guengerich FP, Lapenson DP (1988) Human liver microsomal steroid metabolism: identification of the major microsomal steroid hormone 6 beta-hydroxylase cytochrome P-450 enzyme. Arch Biochem Biophys 263:424–36

42. Nebert DW, Russell DW (2002) Clinical importance of the cytochromes P450. Lancet (London, England) 360:1155–62. https://doi.org/10.1016/S0140-6736(02)11203-7

43. Shi J, Geng MY, Liu CX (2004) Comparative studies of the effects of two novel sugar drug candidates on the CYP 1A2 and CYP 2E1 enzymes in different sexed rats using a “cocktail” approach. Molecules 9:978–987. https://doi.org/10.3390/91100978

44. Boshtam M, Asgary S, Kouhpayeh S, et al (2017) Aptamers Against Pro- and Anti-Inflammatory Cytokines: A Review. Inflammation 40:340–349. https://doi.org/10.1007/s10753-016-0477-1

45. Christensen H, Hermann M (2012) Immunological Response as a Source to Variability in Drug Metabolism and Transport. Front Pharmacol 3:8. https://doi.org/10.3389/fphar.2012.00008

46. Shah P, Guo T, Moore DD, Ghose R (2014) Role of constitutive androstane receptor in toll-like receptor-mediated regulation of gene expression of hepatic drug-metabolizing enzymes and transporters. Drug Metab Dispos 42:172–181. https://doi.org/10.1124/dmd.113.053850

47. Aitken AE, Morgan ET (2007) Gene-specific effects of inflammatory cytokines on cytochrome P450 2C, 2B6 and 3A4 mRNA levels in human hepatocytes. Drug Metab Dispos 35:1687–1693. https://doi.org/10.1124/dmd.107.015511

48. Aitken AE, Richardson TA, Morgan ET (2006) Regulation of Drug-Metabolizing Enzymes and Transporters in Inflammation. Annu Rev Pharmacol Toxicol 46:123–149. https://doi.org/10.1146/annurev.pharmtox.46.120604.141059

49. De-Oliveira ACAX, Poça KS, Totino PRR, Paumgartten FJR (2015) Modulation of Cytochrome P450 2A5 Activity by Lipopolysaccharide: Low-Dose

Effects and Non-Monotonic Dose-Response Relationship. PLoS One 10:e0117842. https://doi.org/10.1371/journal.pone.0117842

50. Schuck RN, Zha W, Edin ML, et al (2014) The Cytochrome P450 Epoxygenase Pathway Regulates the Hepatic Inflammatory Response in Fatty Liver Disease. PLoS One 9:e110162. https://doi.org/10.1371/journal.pone.0110162

51. Raffaele M, Bellner L, Singh SP, et al (2019) Epoxyeicosatrienoic intervention improves NAFLD in leptin receptor deficient mice by an increase in HO-1-PGC1α mitochondrial signaling. Exp Cell Res 380:180–187. https://doi.org/10.1016/j.yexcr.2019.04.029

52. Yeboah MM, Hye Khan MA, Chesnik MA, et al (2018) Role of the cytochrome P-450/ epoxyeicosatrienoic acids pathway in the pathogenesis of renal dysfunction in cirrhosis. Nephrol Dial Transplant 33:1333–1343. https://doi.org/10.1093/ndt/gfx354

53. Zhang C, Booz GW, Yu Q, et al (2018) Conflicting roles of 20-HETE in hypertension and renal end organ damage. Eur J Pharmacol 833:190–200. https://doi.org/10.1016/j.ejphar.2018.06.010

54. Wu K-C, Lin C-J (2019) The regulation of drug-metabolizing enzymes and membrane transporters by inflammation: Evidences in inflammatory diseases and age-related disorders. J Food Drug Anal 27:48–59. https://doi.org/10.1016/j.jfda.2018.11.005

55. Božina N, Bradamante V, Lovrić M (2009) Genetic polymorphism of metabolic enzymes P450 (CYP) as a susceptibility factor for drug response, toxicity, and cancer risk. Arh Hig Rada Toksikol 60:217–242. https://doi.org/10.2478/10004-1254-60-2009-1885

56. Venitt S (1994) Mechanisms of carcinogenesis and individual susceptibility to cancer. Clin Chem 40:1421–5

57. Nebert DW (1991) Role of genetics and drug metabolism in human cancer risk. Mutat Res 247:267–81

58. Shaw G (2013) Polymorphism and single nucleotide polymorphisms (SNPs). BJU Int 112:664–665. https://doi.org/10.1111/bju.12298

59. Mochizuki J, Murakami S, Sanjo A, et al (2005) Genetic polymorphisms of cytochrome P450 in patients with hepatitis C virus-associated hepatocellular carcinoma. J Gastroenterol Hepatol 20:1191–1197. https://doi.org/10.1111/j.1440-1746.2005.03808.x

60. Agundez J a G (2004) Cytochrome P450 gene polymorphism and cancer. Curr Drug Metab 5:211–24. https://doi.org/10.2174/1389200043335621

61. Go RE, Hwang KA, Choi KC (2015) Cytochrome P450 1 family and cancers. J Steroid Biochem Mol Biol 147:24–30. https://doi.org/10.1016/j.jsbmb.2014.11.003

62. Ruparelia KC, Zeka K, Ijaz T, et al (2018) The Synthesis of Chalcones as Anticancer Prodrugs and their Bioactivation in CYP1 Expressing Breast Cancer Cells. Med Chem (Los Angeles) 14:322–332. https://doi.org/10.2174/1573406414666180112120134

63. Johnson AL, Edson KZ, Totah RA, Rettie AE (2015) Cytochrome P450 ω-Hydroxylases in Inflammation and Cancer. In: Advances in Pharmacology, 1st ed. Elsevier Inc., pp 223–262

64. Jarrar YB, Lee S-J (2019) Molecular Functionality of Cytochrome P450 4 (CYP4) Genetic Polymorphisms and Their Clinical Implications. Int J Mol Sci 20:4274. https://doi.org/10.3390/ijms20174274

65. Wang P, Zhang H, Zhang Z, et al (2015) Association of the CYP24A1-rs2296241 polymorphism of the vitamin D catabolism enzyme with hormone-related cancer risk: a meta-analysis. Onco Targets Ther 8:1175–1183. https://doi.org/10.2147/OTT.S80311

66. Xiao Z, Shen J, Zhang L, et al (2018) Therapeutic targeting of noncoding RNAs in hepatocellular carcinoma: Recent progress and future prospects (Review). Oncol Lett 15:3395–3402. https://doi.org/10.3892/ol.2018.7758

67. Bray F, Ferlay J, Soerjomataram I, et al (2018) Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 68:394–424. https://doi.org/10.3322/caac.21492

68. Gao J, Zhou J, He X-P, et al (2016) Changes in cytochrome P450s-mediated drug clearance in patients with hepatocellular carcinoma in vitro and in vivo : a bottom-up approach. Oncotarget 7:28612–28623. https://doi.org/10.18632/oncotarget.8704

69. Zhou J, Wen Q, Li S, et al (2016) Significant change of cytochrome P450s activities in patients with hepatocellular carcinoma. Oncotarget 7:50612–50623. https://doi.org/10.18632/oncotarget.9437

70. Tsunedomi R, Iizuka N, Hamamoto Y, et al (2005) Patterns of expression of cytochrome P450 genes in progression of hepatitis C virus-associated hepatocellular carcinoma. Int J Oncol 27:661–667

71. Zhang D, Lou J, Zhang X, et al (2017) Hyperhomocysteinemia results from and promotes hepatocellular carcinoma via CYP450 metabolism by CYP2J2 DNA methylation. Oncotarget 8:15377–15392. https://doi.org/10.18632/oncotarget.14165

72. Samonakis DN, Koutroubakis IE, Sfiridaki A, et al (2004) Hypercoagulable states in patients with hepatocellular carcinoma. Dig Dis Sci 49:854–858. https://doi.org/10.1023/B:DDAS.0000030099.13397.28

73. Sun CF, Haven TR, Wu TL, et al (2002) Serum total homocysteine increases with the rapid proliferation rate of tumor cells and decline upon cell death: A potential new tumor marker. Clin Chim Acta 321:55–62. https://doi.org/10.1016/S0009-8981(02)00092-X

74. Yan T, Lu L, Xie C, et al (2015) Severely Impaired and Dysregulated Cytochrome P450 Expression and Activities in Hepatocellular Carcinoma: Implications for Personalized Treatment in Patients. Mol Cancer Ther 14:2874–2886. https://doi.org/10.1158/1535-7163.MCT-15-0274

75. Blackburn HL, Ellsworth DL, Shriver CD, Ellsworth RE (2015) Role of cytochrome P450 genes in breast cancer etiology and treatment: effects on estrogen biosynthesis, metabolism, and response to endocrine therapy. Cancer Causes Control 26:319–332. https://doi.org/10.1007/s10552-014-0519-7

76. Cardoso R, Lacerda P, Costa P, et al (2017) Estrogen Metabolism-Associated CYP2D6 and IL6-174G/C Polymorphisms in Schistosoma haematobium Infection. Int J Mol Sci 18:2560. https://doi.org/10.3390/ijms18122560

77. Oyama T (2005) Immunohistochemical evaluation of cytochrome P450 (CYP) and p53 in breast cancer. Front Biosci 10:1156. https://doi.org/10.2741/1608

78. Floriano-Sanchez E, Rodriguez NC, Bandala C, et al (2014) CYP3A4 Expression in Breast Cancer and its Association with Risk Factors in Mexican Women. Asian Pacific J Cancer Prev 15:3805–3809. https://doi.org/10.7314/APJCP.2014.15.8.3805

79. Vaclavikova R, Hubackova M, Stribrna-Sarmanova J, et al (2007) RNA expression of cytochrome P450 in breast cancer patients. Anticancer Res 27:4443–50

80. Parada H, Steck SE, Cleveland RJ, et al (2017) Genetic polymorphisms of phase I metabolizing enzyme genes, their interaction with lifetime grilled and smoked meat intake, and breast cancer incidence. Ann Epidemiol 27:208-214.e1. https://doi.org/10.1016/j.annepidem.2016.11.005

81. Masson LF, Sharp L, Cotton SC, Little J (2005) Cytochrome P-450 1A1 gene polymorphisms and risk of breast cancer: a HuGE review. Am J Epidemiol 161:901–915. https://doi.org/10.1093/aje/kwi121

82. Anttila S, Raunio H, Hakkola J (2011) Cytochrome P450-mediated pulmonary metabolism of carcinogens: Regulation and cross-talk in lung carcinogenesis. Am J Respir Cell Mol Biol 44:583–590. https://doi.org/10.1165/rcmb.2010-0189RT

83. Hukkanen J, Pelkonen O, Hakkola J, Raunio H (2002) Expression and regulation of xenobiotic-metabolizing cytochrome P450 (CYP) enzymes in human lung. Crit. Rev. Toxicol. 32:391–411

84. Oyama T, Sugio K, Isse T, et al (2008) Expression of cytochrome P450 in non-small cell lung cancer. Front Biosci 13:5787–93

85. Song N (2001) CYP 1A1 polymorphism and risk of lung cancer in relation to tobacco smoking: a case-control study in China. Carcinogenesis 22:11–16. https://doi.org/10.1093/carcin/22.1.11

86. Fujita K (2006) Cytochrome P450 and Anticancer Drugs. Curr Drug Metab 7:23–37. https://doi.org/10.2174/138920006774832587

87. Reis M (2006) Farmacogenética aplicada ao câncer. Quimioterapia individualizada e especificidade molecular. Medicina (B Aires) 39:577–586. https://doi.org/10.11606/issn.2176-7262.v39i4p577-586

88. Ingelman-Sundberg M (2004) Pharmacogenetics of cytochrome P450 and its applications in drug therapy: the past, present and future. Trends Pharmacol Sci 25:193–200. https://doi.org/10.1016/j.tips.2004.02.007

89. Tsuji D, Ikeda M, Yamamoto K, et al (2016) Drug-related genetic polymorphisms affecting severe chemotherapy-induced neutropenia in breast cancer patients. Medicine (Baltimore) 95:e5151. https://doi.org/10.1097/MD.0000000000005151

90. Ahmed EM, EL-Maraghy SA, Teleb ZA, Shaheen AA (2014) Pretreatment with turmeric modulates the inhibitory influence of cisplatin and

paclitaxel on CYP2E1 and CYP3A1/2 in isolated rat hepatic microsomes. Chem Biol Interact 220:25–32. https://doi.org/10.1016/j.cbi.2014.05.007

91. Kostrubsky VE, Lewis LD, Strom SC, et al (1998) Induction of Cytochrome P4503A by Taxol in Primary Cultures of Human Hepatocytes. Arch Biochem Biophys 355:131–136. https://doi.org/10.1006/abbi.1998.0730

92. Kostrubsky VE, Ramachandran V, Venkataramanan R, et al (1999) The use of human hepatocyte cultures to study the induction of cytochrome P-450. Drug Metab Dispos 27:887–894. https://doi.org/10.1109/MMCS.1997.609753

93. Li J, Li D, Tie C, et al (2015) Cisplatin-mediated cytotoxicity through inducing CYP4A 11 expression in human renal tubular epithelial cells. J Toxicol Sci 40:895–900. https://doi.org/10.2131/jts.40.895

94. Chang TK, Weber GF, Crespi CL, Waxman DJ (1993) Differential activation of cyclophosphamide and ifosphamide by cytochromes P-450 2B and 3A in human liver microsomes. Cancer Res 53:5629–37

95. Singh MS, Francis PA, Michael M (2011) Tamoxifen, cytochrome P450 genes and breast cancer clinical outcomes. The Breast 20:111–118. https://doi.org/10.1016/j.breast.2010.11.003

96. Thuy Phuong NT, Kim JW, Kim J-A, et al (2017) Role of the CYP3A4-mediated 11,12-epoxyeicosatrienoic acid pathway in the development of tamoxifen-resistant breast cancer. Oncotarget 8:71054–71069. https://doi.org/10.18632/oncotarget.20329

97. Martinez VG, O'Connor R, Liang Y, Clynes M (2008) CYP1B1 expression is induced by docetaxel: Effect on cell viability and drug resistance. Br J Cancer 98:564–570. https://doi.org/10.1038/sj.bjc.6604195

98. McFadyen MCE, McLeod HL, Jackson FC, et al (2001) Cytochrome P450 CYP1B1 protein expression: A novel mechanism of anticancer drug resistance. Biochem Pharmacol 62:207–212. https://doi.org/10.1016/S0006-2952(01)00643-8

99. Brockdorff BL, Skouv J, Reiter BE, Lykkesfeldt AE (2000) Increased expression of cytochrome p450 1A1 and 1B1 genes in anti-estrogen-resistant human breast cancer cell lines. Int J Cancer 88:902–906. https://doi.org/10.1002/1097-0215(20001215)88:6<902::AID-IJC10>3.0.CO;2-C

100. Horley NJ, Beresford KJM, Chawla T, et al (2017) Discovery and characterization of novel CYP1B1 inhibitors based on heterocyclic chalcones:

Overcoming cisplatin resistance in CYP1B1-overexpressing lines. Eur J Med Chem 129:159–174. https://doi.org/10.1016/j.ejmech.2017.02.016

101. Abraham JE, Maranian MJ, Driver KE, et al (2010) CYP2D6 gene variants: association with breast cancer specific survival in a cohort of breast cancer patients from the United Kingdom treated with adjuvant tamoxifen. Breast Cancer Res 12:R64. https://doi.org/10.1186/bcr2629

102. Blancas I, Rodriguez Gonzalez CJ, Muñoz-Serrano AJ, et al (2018) Influence of CYP2D6 polymorphism in the outcome of breast cancer patients undergoing tamoxifen adjuvant treatment. J Clin Oncol 36:e12521–e12521. https://doi.org/10.1200/JCO.2018.36.15_suppl.e12521

103. Lan B, Ma F, Zhai X, et al (2018) The relationship between the CYP2D6 polymorphisms and tamoxifen efficacy in adjuvant endocrine therapy of breast cancer patients in Chinese Han population. Int J Cancer 143:184–189. https://doi.org/10.1002/ijc.31291

104. Chan CWH, Law BMH, So WKW, et al (2020) Pharmacogenomics of breast cancer: highlighting CYP2D6 and tamoxifen. J Cancer Res Clin Oncol 146:1395–1404. https://doi.org/10.1007/s00432-020-03206-w

105. Mwinyi J, Vokinger K, Jetter A, et al (2014) Impact of variable CYP genotypes on breast cancer relapse in patients undergoing adjuvant tamoxifen therapy. Cancer Chemother Pharmacol 73:1181–1188. https://doi.org/10.1007/s00280-014-2453-5

106. Hassan M, Nilsson C, Olsson H, et al (1999) The influence of interferon-alpha on the pharmacokinetics of cyclophosphamide and its 4-hydroxy metabolite in patients with multiple myeloma. Eur J Haematol 63:163–170

107. Islam M, Frye RF, Richards TJ, et al (2002) Differential effect of IFNalpha-2b on the cytochrome P450 enzyme system: a potential basis of IFN toxicity and its modulation by other drugs. Clin Cancer Res 8:2480–7

108. Egbelakin A, Ferguson MJ, MacGill EA, et al (2011) Increased risk of vincristine neurotoxicity associated with low CYP3A5 expression genotype in children with acute lymphoblastic leukemia. Pediatr Blood Cancer 56:361–367. https://doi.org/10.1002/pbc.22845

109. Deenen MJ, Cats A, Beijnen JH, Schellens JHM (2011) Part 2: Pharmacogenetic Variability in Drug Transport and Phase I Anticancer Drug Metabolism. Oncologist 16:820–834. https://doi.org/10.1634/theoncologist.2010-0259

110. Marre F, Sanderink GJ, De Sousa G, et al (1996) Hepatic biotransformation of docetaxel (Taxotere®) in vitro: Involvement of the CYP3A subfamily in humans. Cancer Res 56:1296–1302

111. Rodriguez-Antona C, Ingelman-Sundberg M (2006) Cytochrome P450 pharmacogenetics and cancer. Oncogene 25:1679–1691. https://doi.org/10.1038/sj.onc.1209377

112. Relling M V, Nemec J, Schuetz EG, et al (1994) O-demethylation of epipodophyllotoxins is catalyzed by human cytochrome P450 3A4. Mol Pharmacol 45:352–8

113. Shet MS, McPhaul M, Fisher CW, et al (1997) Metabolism of the antiandrogenic drug (Flutamide) by human CYP1A2. Drug Metab Dispos 25:1298–303

114. Makowsky GS (2015) Advances in clinical chemistry, Volume 71. Elsevier, 225 Wyman Street, Waltham, MA 02451, USA 525 B Street, Suite 1800, San Diego, CA 92101-4495, USA 125 London Wall, London, EC2Y 5AS, UK The Boulevard, Langford Lane, Kidlington, Oxford OX5 1GB, UK

115. Chugh R, Wagner T, Griffith KA, et al (2007) Assessment of ifosfamide pharmacokinetics, toxicity, and relation to CYP3A4 activity as measured by the erythromycin breath test in patients with sarcoma. Cancer 109:2315–2322. https://doi.org/10.1002/cncr.22669

116. Peng B, Lloyd P, Schran H (2005) Clinical Pharmacokinetics of Imatinib. Clin Pharmacokinet 44:879–894. https://doi.org/10.2165/00003088-200544090-00001

117. Cresteil T, Monsarrat B, Alvinerie P, et al (1994) Taxol metabolism by human liver microsomes: identification of cytochrome P450 isozymes involved in its biotransformation. Cancer Res 54:386–92

118. Rahman A, Korzekwa KR, Grogan J, et al (1994) Selective biotransformation of taxol to 6 alpha-hydroxytaxol by human cytochrome P450 2C8. Cancer Res 54:5543–6

119. Gong L, Giacomini MM, Giacomini C, et al (2017) PharmGKB summary. Pharmacogenet Genomics 27:240–246. https://doi.org/10.1097/FPC.0000000000000279

120. Kobayakawa M, Kojima Y (2011) Tegafur/gimeracil/oteracil (S-1) approved for the treatment of advanced gastric cancer in adults when given in

combination with cisplatin: a review comparing it with other fluoropyrimidine-based therapies. Onco Targets Ther 4:193. https://doi.org/10.2147/OTT.S19059

121. Sulkes A, Benner SE, Canetta RM (1998) Uracil-ftorafur: an oral fluoropyrimidine active in colorectal cancer. J Clin Oncol 16:3461–3475. https://doi.org/10.1200/JCO.1998.16.10.3461

122. Chhetri P (2016) Current Development of Anti-Cancer Drug S-1. J Clin DIAGNOSTIC Res 10:XE01–XE05. https://doi.org/10.7860/JCDR/2016/19345.8776

3 ARTIGO CIENTÍFICO 2 – “INFLUENCE OF RED WINE POLYSACCHARIDES IN CYP ENZYMES AND INFLAMMATORY PARAMETERS IN TUMOR MODELS”

Influence of red wine polysaccharides on CYP enzymes and inflammatory parameters in tumor models

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3.1 ABSTRACT

Proinflammatory cytokines present an important role in carcinogenesis and in regulation of cytochrome P enzymes (CYPs) in tumor and liver. The soluble fraction of polysaccharides from cabernet franc red wine (SFP) has antitumoral effect by modulation of the immune system. The hypothesis that SFP can regulate CYPs was tested *in vitro* (HepG2 cells) and *in vivo* (Walker-256 tumor-bearing rats). Vincristine (Vin) was used as a positive control. SFP was used in the protocols of *a)* solid tumor, *b)* liquid tumor and *c)* chemopreventive solid tumor. SFP reduced solid tumor development in both protocols, but did not inhibit the liquid tumor. SFP reduced total CYP levels in *a* and *b* protocols, as well as the gene expression of *Cyp1a1* and *Cyp2e1* in rats and *CYP1A2* in HepG2 cells. An increase of NAG activity in all treated rats, and in TNF-α levels in *a* protocol in Veh, SFP and Vin group was observed. Protocol *c* did not modify the CYP level in liver and intestine, nor the NAG and MPO in liver. Additionally, *in vitro* digestion and NMR analyses suggest that SFP was slighted modified along the gastrointestinal system. In conclusion, SFP inhibits CYPs *in vivo* and *in vitro*, probably as a result of its immunomodulatory effect.

Keywords: Cytochrome P450; inflammation; Walker-256; cancer; polysaccharides; red wine

Abbreviations: Alanine aminotransferase, ALT; Analysis of variance, ANOVA; Aspartate aminotransferase, AST; Cisplatin, Cis; Ciplatin + SFP, CS; Cisplatin + Vincristine, CV; Cisplatin + Vincristine + SFP, CVS; Cytochrome P450, CYP; Deuterium oxide, D_2O; Ethylenediaminetetraacetic acid, EDTA; Enzyme linked immunosorbent assay, ELISA; Fetal bovine serum, FBS; Glyceraldehyde 3-phosphate dehydrogenase, GAPDH; Interleukin-1, IL-1; Interleukin-6, IL-6; Inferferons, INFs; Intraperitoneal, i.p.; Myeloperoxidase, MPO; N-acetylglucosaminidase, NAG; Pathogen-associated molecular patterns, AMPs; Phosphate buffer, PBS; Quantitative polymerase chain reaction, qPCR; Standard error of the mean, SEM; Soluble fraction of polysaccharides from cabernet franc red wine, SFP; Subcutaneously, s.c.; Toll-like receptors, TLRs; Trimethylsilyl propionic

acid, TMSP; Tumor necrosis factor-α, TNF-α; Vehicle, Veh; Vincristine, Vin; Vincristine + SFP, VS.

3.2 INTRODUCTION

The enzymatic system cytochrome P450 (CYPs) corresponds to membrane-bound hemoprotein, responsible for xenobiotics detoxification, cellular metabolism and homeostasis. The interindividual variability of CYPs in drug disposition plays a pivotal role in therapeutic responses or adverse effects of drug-drug interactions. These differences include induction or inhibition mechanisms, stimulated by xenobiotics and endogenous substances, which can activate or block the transcription of CYPs enzymes; besides epigenetic changes and genetic polymorphisms, since the CYPs family presents different alleles in the population (1–3).

During inflammation responses or infectious diseases, proinflammatory cytokines like interleukin (IL)-1 and IL-6, interferons (IFNs) and tumor necrosis factor-α (TNF-α) are induced by monocytes, macrophages and stromal cells. These cytokines are produced when pathogen-associated molecular patterns (PAMPs) act in toll-like receptors (TLRs), which in the liver are present in Kupffer cells and hepatocytes. Therefore, inflammatory responses in the liver are associated with TLRs, which act on hepatocytes to regulate the expression of genes, such as the CYPs genes (4). TNF-α decreases CYP activity and promotes inhibition of the most important CYPs related to drug metabolism (5), both CYP3A4 and CYP2D6. Consequently, these enzymes respond to the inflammatory process provided by diseases, such as cancer.

Cancer is caused by key genes-mutated cells that confer uncontrollable multiplication and cell survival capacity. In these conditions, CYP regulation can be altered. CYPs are considered key enzymes in cancer formation and treatment, because they mediate the activation of precarcinogens and both inactivation and activation of anticancer drugs (3). Several evidences are associated with CYP polymorphisms and cancer risk, essentially by the polymorphisms of a single nucleotide, gene duplications, and deletions. The gene polymorphisms of CYPs in cancer development have been investigated, and the most relevant are CYP1A1, CYP1A2, CYP2D6, CYP2E1 and CYP3A4 (6). Then, investigation of drug effects upon these enzymes is necessary for pharmacology safety evaluations and

prediction of inter-drug interactions. These points become important for the design of more suitable drug administration protocols (7).

Accordingly, new substances have been studied, for antitumoral effects and less side effects to the patients. In this context, polysaccharides are interesting candidates, since they promote modulation of biological responses, essentially in immune systems. The soluble fraction of polysaccharides extracted from cabernet franc red wine (SFP), has demonstrated a relevant antitumor effect (8). The SFP is responsible for modulating the immune system, increasing the levels of TNF-α and inducing necroptosis in tumor cells (8). Because of this modulation, it is possible that SFP causes inhibition of the CYPs, and therefore impairment of other chemotherapeutic agents administered in conjunction with SFP.

The aim of this study was to evaluate the effects of SFP, alone or in combination with other chemotherapeutic agent, on cytochrome P450 enzymes and in inflammatory parameters. For this, the Walker-256 tumor, a rat model of carcinoma, was used *in vivo*. This tumor has similarity with several tumors in human patients, since it can induce cachexia, inflammation, liberation of cytokines, and inhibition of hepatic CYP activity (9). HepG2 cells, the human hepatoma cell line most commonly used in drug metabolism and hepatotoxicity studies, was also cultured with SFP. Additionally, the *in vitro* digestion of SFP to simulate the human gastrointestinal tract was performed to determine the SFP digestion when used by oral via.

3.3 MATERIAL AND METHODS

3.3.1 Cell culture

The hepatocellular carcinoma cells HepG2 obtained from ATCC were used in *in vitro* experiments. The HepG2 cells were cultivated in high-glucose DMEM supplemented with 10% fetal bovine serum (FBS) and 100 U/mL penicillin, at 37ºC in a humidified atmosphere containing 5% CO_2. Cells were passaged every 2-3 days at confluence of about 70-80% and plated at a density of 10^6 cells/well. The treatments were divided in nine: DMEM (negative control), 5 µmol Cisplatin (Cis), 50 µmol Vincristine (Vin), 50 µg Soluble Fraction of Polysaccharide (SFP), 5 µmol Cisplatin + 50 µmol Vincristine (CV), 5 µmol Cisplatin + 50 µg SFP (CS), 50 µmol Vincristine + 50 µg SFP (VS), 5 µmol Cisplatin + 50 µmol Vincristine + 50 µg SFP (CVS). These experimental protocols were conducted in triplicates.

3.3.1.1 MTT assay

HepG2 cells are plated at 10^4 cells/well into 96-well culture plates by 24 hours. After, the cells were treated in triplicates with 200 µL of the treatments cited in section 3.3.1 by 24 and 48 hours. The MTT assay was evaluated in 550 nm, to express a cell viability according Reilly et al. (1998). Results are expressed in percentage of viable cells in comparison to the control.

3.3.2 Walker-256 tumor inoculation and posology regimes

The male Wistar rats used in the experiments were obtained from the vivarium of the Federal University of Paraná (UFPR) (Curitiba, Brazil). The animals were housed in a controlled temperature (22 ± 1ºC) with a 12/12h light/dark cycle and free access to water and food.

The maintenance of the Walker-256 cells was through weekly intraperitoneal (i.p.) inoculation at 10^7 cells/rat. The i.p. cells were collected in a solution of ethylenediaminetetraacetic acid (EDTA) (0.5 M, pH 8.0) and suspended in 1.0 mL of PBS (16.5 mM phosphate, 137 mM NaCl and 2.7 mM KCl), after four or five passages, which lasted 3-4 days of the cell growth in ascitic form. The cell viability was verified by the trypan blue method in a Neubauer chamber, then tumor cells were subcutaneously (s.c.) injected in the right hind paw, at 2×10^7 cells/rat, in a final volume of 0.2 mL. Rats were separated into groups (n= 6–8) and treated according to protocols descripted in Figure 1. SFP was prepared at Department of Biochemistry of UFPR. The composition and characterization of SFP were previously reported by our group (8). The experimental protocols in rats were divided in:

a) Solid tumor protocol: treatment was initiated 1 day after the s.c. tumor cells inoculation and was followed for 14 days;

b) Liquid tumor (ascitic) protocol: treatment was initiated 1 day after i.p. tumor cells inoculation and was followed for 5 days;

c) Chemoprevention solid tumor protocol: treatment with SFP 6 mg/kg or distillated water was initiated 14 days before the s.c. tumor cells inoculation (day 0). After, rats were divided in different groups, treated for more 14 days.

In all protocols the SFP was daily dissolved in distilled water (vehicle), just prior to the administration. Vincristine was dissolved in 0.9% saline solution and administered i.p. every 5 days at solid tumor protocol or in the -1 day (one day before inoculation) in liquid tumor protocol. Experimental design and treatment groups are

descripted in Figure 1. The Basal group was composed by healthy rats (no tumor) treated with SFP 60 mg/kg, and Naive group was composed by healthy rats treated with vehicle (distilled water) by oral via.

In the end of the treatment of solid tumor protocol and chemopreventive tumor protocol, the animals were anesthetized with an i.p. injection of ketamine hydrochloride (100 mg/kg) and xylazine hydrochloride (10 mg/kg), for biological material sampling (blood, tumor and liver). The liver and solid tumor were harvested, weighed and frozen (-80ºC) for further analyses. Finally, euthanasia was performed under anesthesia by puncturing the diaphragm. In the ascitic tumor groups, the animals were euthanized by cervical displacement, the liquid tumor was collected, the volume was measured and the cell viability analyzed by Tripan blue method (10).

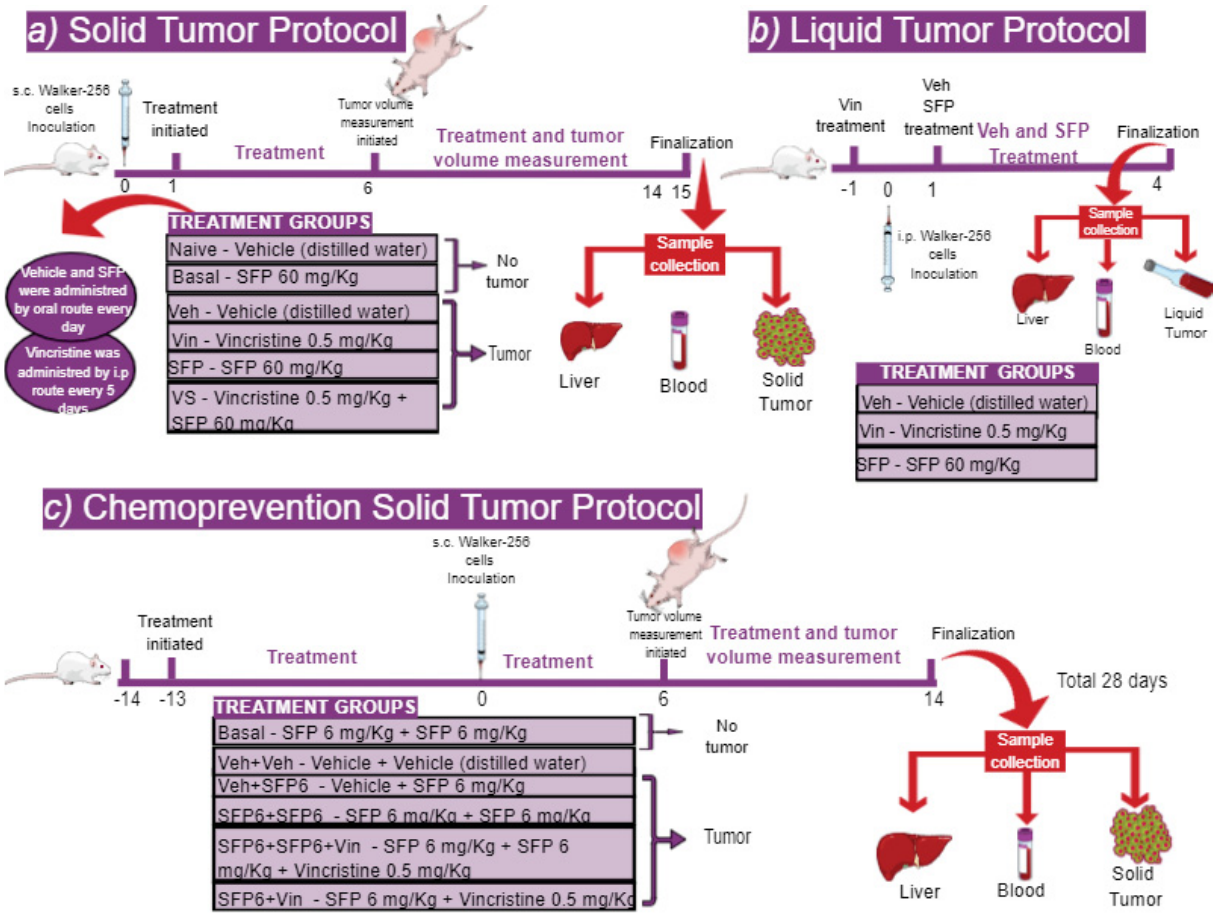


Figure 1 - Experimental design of treatment protocols. Lines and numbers represent the duration of the experiment in days.

3.3.3 Plasmatic biochemistry and hemogram

Blood samples were collected from the abdominal cava vein in heparinized syringes to evaluated hematologic parameters, using a BC2800-Vet (Mindray, Shenzhen, China) automated device. After, the blood samples were submitted to centrifugation at 3,000 rpm for 10 minutes to obtained the plasma, to determinate glucose, alanine aminotransferase (ALT/SGPT) and aspartate aminotransferase (AST/SGOT), by means of commercial kits (Kovalent, Reagelabor, São Paulo, Brazil) using an automated device (Cobas Mira, Roche Diagnostics, Germany).

3.3.4 Measurement of total CYP in liver and intestine tissues

The CYP concentration was measured according to Matsubara (11), by the method of dithionite difference, from the spectrum of “CO-bubbled samples”. The liver and jejunum homogenates were prepared with 10 mg wet tissue/mL in a 50 mM Tris-HCl buffer (pH 7.4), containing 150 mM KCl and 10 mM $MgCl_2$. CO was bubbled through the suspension for about 1 min. The samples were divided in microplates in duplicate and the baseline was recorded. Then 6 µl of sodium dithionite was added to the contents in the sample and the spectrum was obtained after 4 min. The concentration of CYP was determined from the spectrum using the molar extinction (104 $mM^{-1} \cdot cm^{-1}$) for the absorption difference between the peaks in 450 nm and 490 nm (11). The results were expressed in nmol CYP/mg tissue.

3.3.5 Inflammatory parameters

3.3.5.1 Determination of the enzymatic activity of N-acetylglucosaminidase (NAG) and myeloperoxidase (MPO) in liver

The enzyme activity of MPO and NAG indicates neutrophil and macrophage (mononuclear cell) migration, respectively (12,13). Samples of liver were weighed (0.1 g) and homogenized in 0.1% Triton X-100 saline, followed by centrifugation at 10,000 rpm at 4°C for 10 min. The supernatants were used to determine MPO and NAG activity, according to Bradley et al. (12), at 620 nm, and Sánchez & Moreno (13), at 405 nm, respectively.

3.3.5.2 Determination of TNF-α levels in liver

Liver samples were homogenized in phosphate buffer (pH 6.5; 1:10 dilution), and the homogenate was centrifuged at 10,000 rpm for 20 min at 4ºC. The supernatant was used to measure the TNF-α concentrations using an enzyme linked immunosorbent assay (ELISA) kit according to the manufacturer's instructions (Boster Biological Technology, Pleasanton, CA, USA; PeproTech Inc., Cranbury, USA).

3.3.6 Gene expression

RNA extraction was performed in liver samples with TRIzol reagent (Life Technologies Carlsbac, CA, USA), and in HepG2 cells with RNA extracting Kit. The cDNA was prepared from 2 µg RNA in a 20 µL reaction volume according the protocol of the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, ThermoFisher Scientific, Waltham, MA, USA). The quantitative polymerase chain reaction (qPCR) was performed using a Step One Plus thermocycler with the 1x QuantiTect SYBR Green PCR Kit (Qiagen, Germantown, MD, USA). The sequence of the primers (Invitrogen, São Paulo, Brazil), is presented in supplementary Table S1. The results are expressed by relative expression levels of the housekeeper gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH).

3.3.7 SPF digestion *in vitro*

The static *in vitro* digestion model to simulate the human gastrointestinal tract was performed according to studies that were previously reported (14), with some modifications. All solutions used for the experiment were warmed to 37 ± 0.5ºC, and all chemicals were purchased from Sigma-Aldrich (St. Louis, MO). Activities of the mouth, stomach and small intestine were simulated. Digestion started with 100 mg of SFP in 50 mL polyethylene tubes, which received 0.6 mL of artificial saliva [10 mL of KCl (89.6 g/L), 10 mL of KSCN (20 g/L), 10 mL of NaH_2PO_4 (88.8 g/L), 10 mL of Na_2SO_4 (57 g/L), 1.7 mL of NaCl (175.3 g/L), 20 mL of $NaHCO_3$ (84.7 g/L), 8 mL of urea (25 g /L) and 290 mg of α-amylase, completed to 0.5 L and pH adjusted to 6.8], 10 mL of deionized water, 500 U of pepsin, and the pH was adjusted to 2.0 ± 0.2 with HCl 1 M. The material was incubated in a water bath set at 100 rpm orbital rotation (Dubnoff TE-053, TECNAL, Piracicaba, SP, Brazil) and 37ºC for 2 hours, corresponding to the mouth and stomach phases. After incubation, 20 mL of

deionized water, 0.5 mg of pancreatin 4×USP, and 3.1 mg of bile salts were added, and the pH was adjusted to 6.5 ± 0.2 with $NaHCO_3$ 1M. Tubes were incubated again for 2 h, under the same conditions described above. At the end of the process, aliquots were lyophilized to evaluate the SPF bioaccessibility during gastric and duodenal simulated digestion by NMR analyses.

3.3.8 NMR spectroscopy of *in vitro* digested SPF

1D and 2D NMR spectra were obtained with a 600 MHz Bruker spectrometer using 5 mm inverse probehead (Avance III, Bruker, Billerica, Massachusetts, USA). 1D ^{1}H and 600 MHz were collected after a 90° (p1) pulse calibration. The $^{1}H/^{13}C$ chemical shift correlation mapping was finally determined by HSQCed performed at 303 K in deuterium oxide (D_2O). 2D NMR spectra were integrated according cross peak volumes and polysaccharide composition was determined after normalization (15,16). The chemical shifts of the polysaccharide were expressed in δ (ppm) relative to trimethylsilyl propionic acid (TMSP).

3.3.9 Statistical analysis

The statistical analysis was performed using GraphPad Prism 5.0 software (San Diego, CA, USA). The data were analyzed using one- or two-way analysis of variance (ANOVA) followed by Tukey's or Bonferroni's *post hoc* test, respectively. The parameters analyzed in two-way ANOVA were treatment (groups) and time (days of treatment). The criterion for statistical significance was $p < 0.05$. The results are expressed as mean ± standard error of the mean (SEM).

3.4 RESULTS

3.4.1 CYPs expression and cytotoxicity on HepG2 cells

We analyzed the CYPs gene expression in HepG2 cells cultured for 24 hours, under different treatments. All compounds were able to inhibit the gene expression of *CYP1A2* (Fig. 2A). However, the association between cisplatin and SFP (CS group) reversed this inhibitory effect. It is also noteworthy that the association between Vincristine and SFP had an inhibitory effect, but this effect was less expressive than that of both isolated drugs. Interestingly, the effect upon cell viability was also less relevant in the CS group (Fig. 2C, D). Gene expression of *CYP2B6* in HepG2 did not change with the treatments (Fig. 2B).

Cytotoxicity was tested by MTT method in 24 (Fig. 2C) and 48 h (Fig. 2D) to also investigate the effect of SFP alone or in combination with the chemotherapeutics Cisplatin and Vincristine. SFP treatment did not affect the HepG2 cells viability in 24 and 48 h. However, in 24 h Vin, CV, VS and CVS reduced the cells viability, while after 48 h all treatments with the chemotherapeutics, alone or in combination, presented this effect. Instead, SFP reduced the cytotoxicity of Cisplatin.

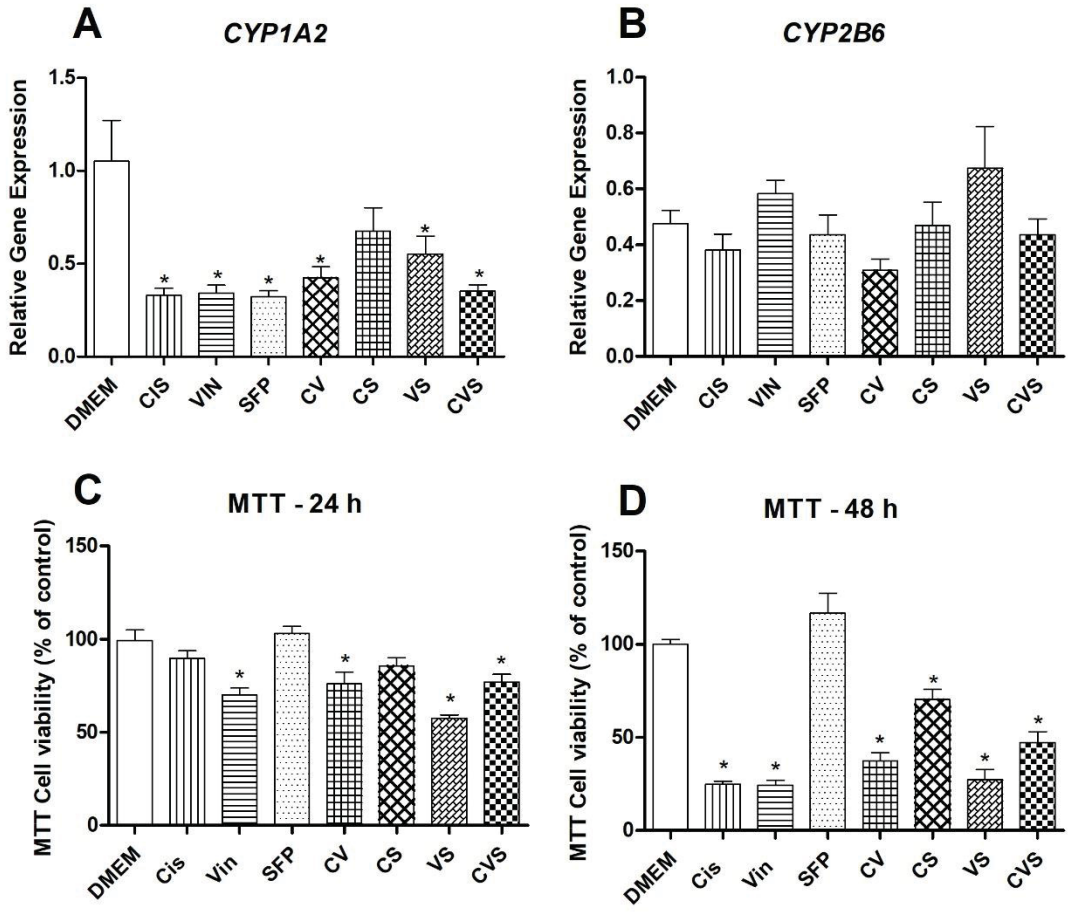


Figure 2 - Gene expression of (A) *CYP1A2* and (B) *CYP2B6*, and cell viability in 24 h (C) and 48 h (D) of HepG2 cells. The cells were treated by 24 or 48 h with DMEM (negative control), 5 µmol Cisplatin (Cis), 50 µmol Vincristine (Vin), 50 µg Soluble Fraction of Polysaccharide (SFP), 5 µmol Cisplatin + 50 µmol Vincristine (CV), 5 µmol Cisplatin + 50 µg SFP (CS), 50 µmol Vincristine + 50 µg SFP (VS), 5 µmol Cisplatin + 50 µmol Vincristine + 50 µg SFP (CVS). The results are expressed in percentage of control (DMEM) and represent the mean ± SEM of three experiments. One-way ANOVA followed by Tukey's multiple comparisons test was used for statistical analysis. Symbol: * significantly different $p < 0.05$ compared with DMEM.

3.4.2 Effects of SFP on Solid tumor and Liquid tumor protocols

3.4.2.1 Antitumor effects of SFP

Walker-256 tumor was visible at day 5 after inoculation, from when the tumor volume measurements were initiated. SFP (SFP 60 mg/kg), VS (SFP 60 mg/kg + Vincristine 0.5 mg/kg) and Vin (Vincristine 0.5 mg/kg) groups presented a significant reduction of tumor volume (Fig. 3A) and tumor weight (Fig. 3B), when compared with the vehicle group. The difference between groups of solid tumor volume was significant from the 10th day of treatment. However, SFP or Vincristine treatment did not reduce the total volume of peritoneal liquid (Fig. 3C) or cell viability in Walker-256 ascitic tumor (Fig. 3D).

Glucose, AST, and ALT were evaluated in plasma (Table S2). Glucose levels increased in Vin and VS group, and AST activity decreased in SFP, Vin and VS group. Additionally, hemogram demonstrated decrease of the lymphocytes count in Vin and VS group, when compared to the vehicle group, and increased of platelets counts (Table S3). On the other hand, SFP treatment did not induce hematological alterations (Table S3).

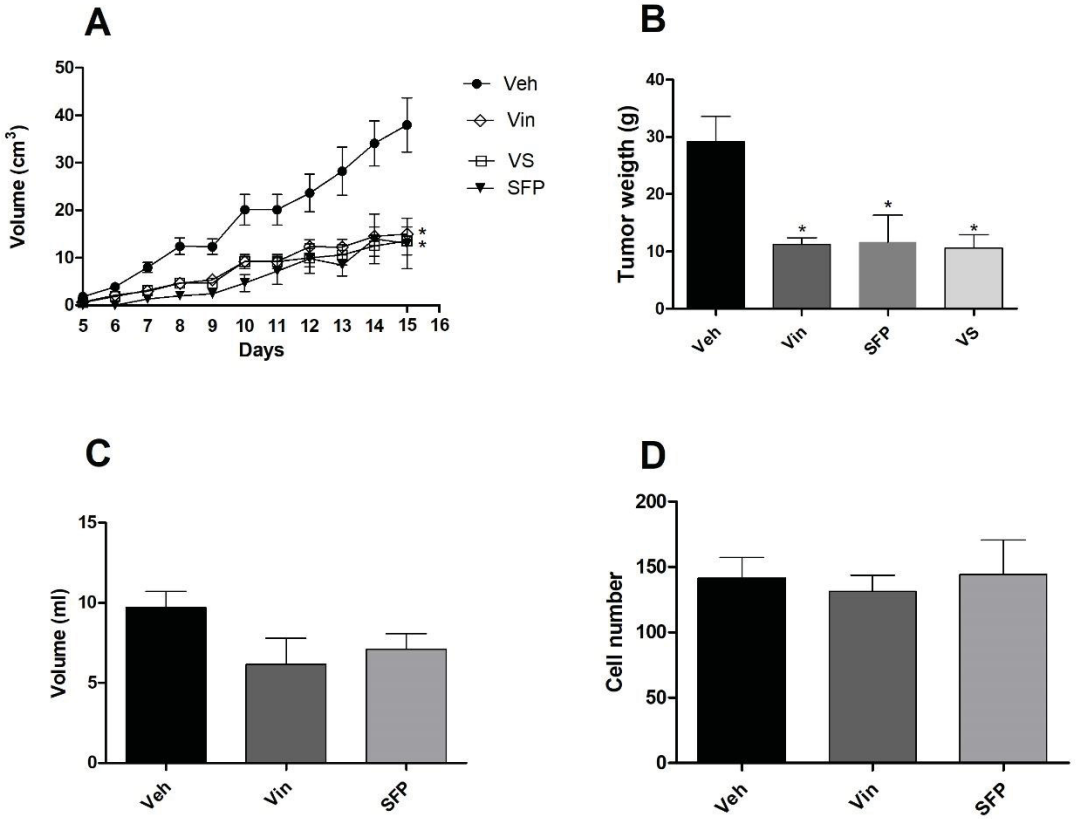


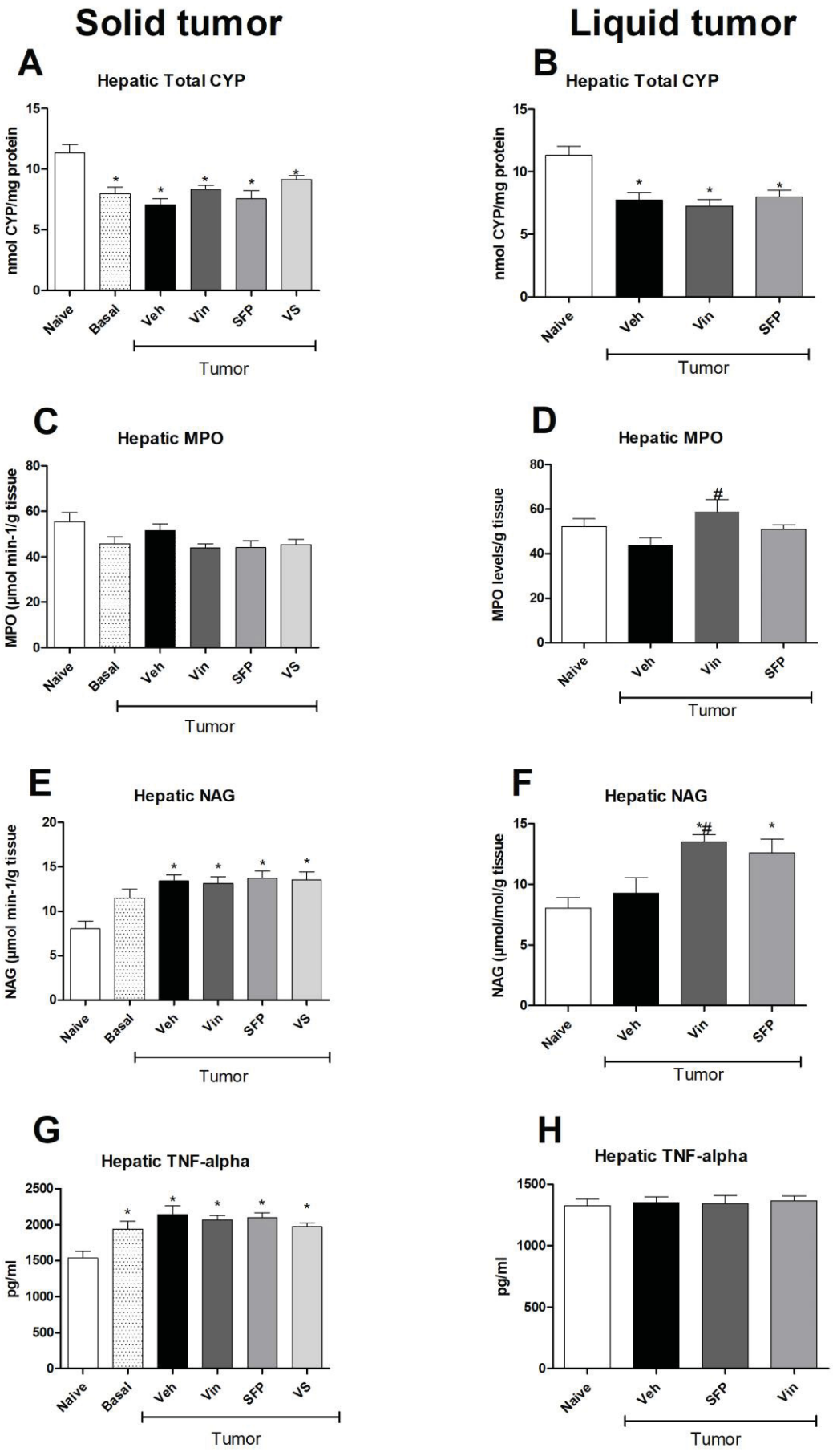
Figure 3 – Antitumor effect of SFP in solid and liquid Walker-256 tumor. Tumor volume (A) and weight (B) of Walker-256 tumor bearing-rats treated with Vehicle (Veh), SFP 60 mg/kg (SFP), SFP 60 mg/kg + Vincristine 0.5 mg/kg (VS) and Vincristine 0.5 mg/kg (Vin), for 14 days. Peritoneal fluid volume (C) and cell viability by Trypan blue (D) of peritoneal Walker-256 tumor bearing-rats treated with Vehicle

(Veh), SFP 60 mg/kg (SFP60) and Vincristine 0.5 mg/kg (Vin), during 5 days. The values are expressed as mean ± S.E.M. of 6-8 rats. The one-way ANOVA followed by Tukey's multiple comparisons test was used in B, C, D, and two-way ANOVA followed by Bonferroni's test were used in A. Symbol: * $p < 0.05$ as compared to the Vehicle group.

3.4.2.2 Total hepatic CYP and inflammatory parameter levels

Animals from both solid (Basal, Veh, Vin, SFP, and VS) and liquid tumor (Veh, Vin, and SFP) protocols presented reduction of hepatic total CYP when compared to Naive group (Fig. 4A; 4B). Inversely, the Basal, Veh, Vin, SFP e VS groups, presented an increase of hepatic NAG in the solid tumor protocol (Fig. 4E), accompanied by an increase in TNF-α levels, when compared to the Naive group (Fig. 4G). MPO enzyme activity was not altered among groups (Fig. 4C).

Regarding the liquid tumor protocol, hepatic NAG activity was increased in Vin and SFP group when compared to the Naive group (Fig. 4F). In the same way, MPO activity was increased in Veh, Vin and SFP groups, when compared to the Naive group (Fig. 4D). Vin treatment resulted in higher activity of NAG (44%) and MPO (34%) compared to the Veh group, demonstrating that this treatment induced inflammatory cell migration into the liver. TNF-α levels were not altered between groups in liquid tumor protocol (Fig. 4H).


Solid tumor
Liquid tumor
A
Hepatic Total CYP
nmol CYP/mg protein
Naive
Basal
Veh
Vin
SFP
VS
Tumor
B
Hepatic Total CYP
nmol CYP/mg protein
Naive
Veh
Vin
SFP
Tumor
C
Hepatic MPO
MPO (µmol min-1/g tissue
Naive
Basal
Veh
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Tumor
D
Hepatic MPO
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Vin
Tumor

Figure 4 - Measurement of total CYP and inflammatory parameters in liver tissue. Total CYP (A, B), Myeloperoxidase (C, D), N-acetylglucosaminidase (E, F) and TNF –alpha (G, H) in solid and liquid tumor models. Samples were obtained from rats without tumor, treated with vehicle (Naive) or SFP 60 mg/kg (Basal); and Walker-256 tumor bearing-rats treated with vehicle (Veh), Vincristine 0.5 mg/kg (Vin), SFP 60 mg/kg (SFP) and Vincristine 0.5 mg/kg + SFP 60 mg/kg (VS) during 14 days in the solid tumor model; and Walker-256 tumor bearing-rats treated with vehicle (Veh), Vincristine 0.5 mg/kg (Vin) or SFP 60 mg/kg (SFP) during 5 days in the liquid tumor model. Values are expressed as mean ± S.E.M. of 6-8 rats. Statistical comparison was performed using One-way ANOVA followed by Tukey's multiple comparisons test. Symbol: * $p < 0.05$ as compared to the Naive group; # $p < 0.05$ as compared to the Vehicle group.

3.4.2.3 Hepatic gene expression in rats

Gene expression was performed to indicate which CYP isoforms could be inhibited by the treatments in tumor-bearing rats. Considering the main enzymes involved in carcinogenesis and drug metabolism, we verified the gene expression of *Cyp3a9*, *Cyp2e1, Cyp2d4* and *Cyp1a1*. CYP3A9 was evaluated because it shared an elevated homology with human CYP3A4 (17), while CYP2D4 is an analogue to the human CYP2D6. The Basal, Vin and VS groups presented an inhibitory effect on *Cyp3a9* gene expression (Fig. 5A), when compared to Naive group. Similarly, the *Cyp2e1* (Fig. 5B) was inhibited in Basal, SFP, Vin and VS groups, when compared to naive. However, no changes were detected in *Cyp2d4* expression (Fig. 5C). *Cyp1a1*, was induced by the tumor (Veh) when compared to the Naive group. Moreover, SFP treatment promoted an inhibition of *Cyp1a1* mRNA expression, in both groups with (SFP) and without tumor (Basal), when compared to Veh group (Fig. 5D).

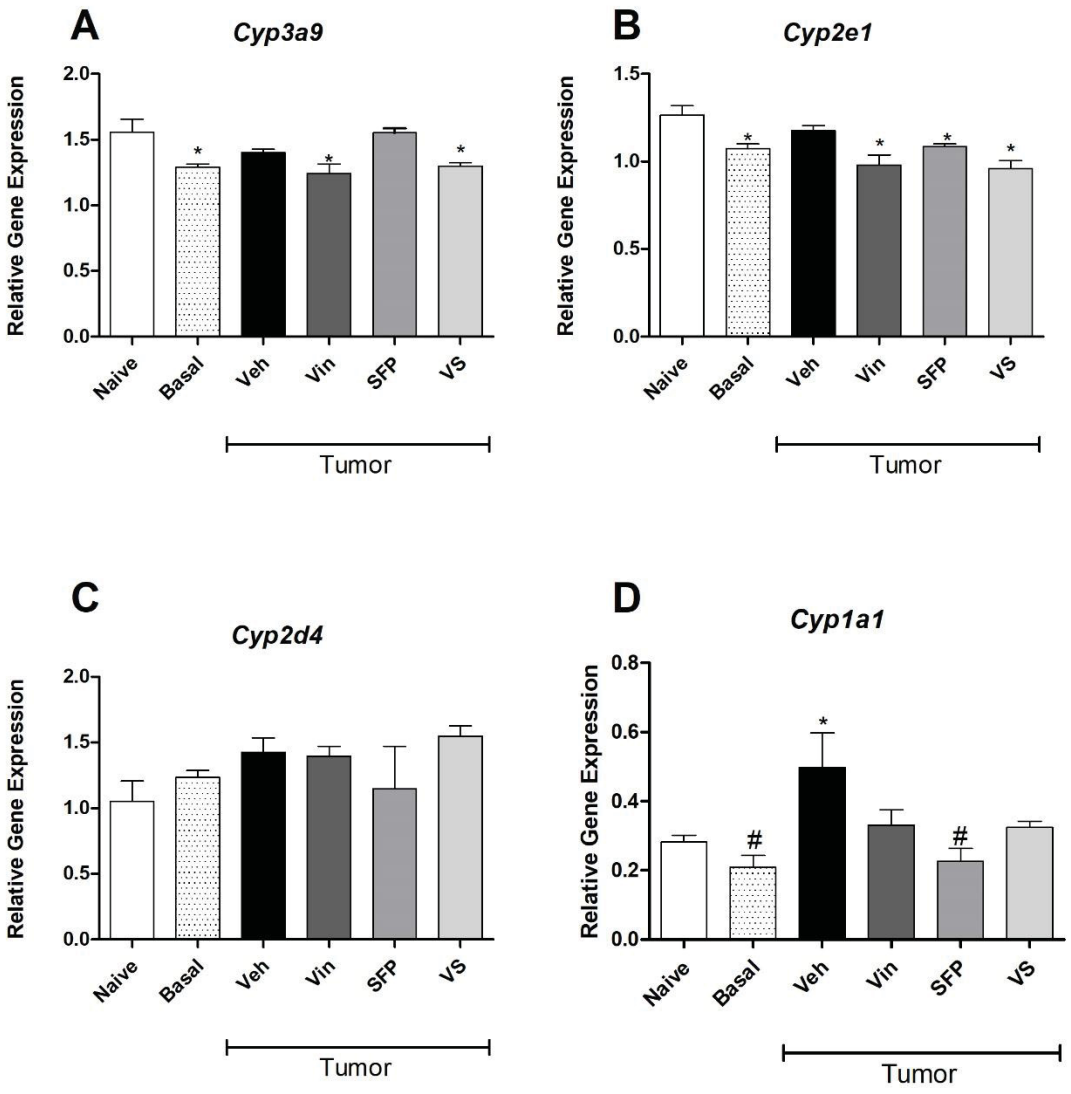

Figure 5 – Hepatic gene expression of (A) *Cyp3a9*, (B) *Cyp2e1*, (C) *Cyp2d4* and (D) *Cyp1a1* of rats without tumor treated with vehicle (Naive) or SFP 60 mg/kg (Basal); and Walker-256 solid tumor bearing-rats treated with vehicle (Veh), Vincristine 0.5 mg/kg (Vin), SFP 60 mg/kg (SFP) and Vincristine 0.5 mg/kg + SFP 60 mg/kg (Vin + SFP) for 14 days. The values are expressed in mean ± S.E.M. of 6 rats, demonstrated as the *Gapdh* relative expression. One-way ANOVA followed by Tukey's multiple comparisons test was used to statistical analyze. Symbol: * $p < 0.05$ compared to the Naive group; # $p < 0.05$ compared to the Vehicle group.

3.4.3 Effects of SFP on Chemoprevention solid tumor protocol

3.4.3.1 Effects of chemopreventive treatment in CYP and inflammatory parameters

Considering the inhibitory effects on CYP enzymes observed with the antitumor dose of 60 mg/kg SFP, we analyzed the effects of these polysaccharides in lower dose and longer treatment on CYP enzymes. For this, the animals were pre-treated with 6 mg/kg SFP for a period of 14 days before receiving the inoculation of

Walker-256 cells, followed for the next 14 days by treatment after tumor inoculation. After this period, the previous treatment with lower dose of SFP resulted in an antitumor effect in all groups studied (SFP6+Veh; SFP6+SFP6; SFP6+SFP6+Vin; SFP6+Vin), as showed in Fig 6A and 6B. However, the pretreatment did not decrease the CYP levels (Fig. 6C). Only the Veh+Veh group showed inhibition in total CYP levels when compared with the Naive group. The groups that received SFP 6 mg/kg for a prolonged period lost the inhibitory effect upon the CYP enzymes, including the group treated with Vincristine. In the presence of solid tumor the treatment with SFP (SFP+Veh; SFP+SFP) did not change the hepatic NAG activity, while the reduction of NAG activity was clearly related with the vincristine treatment (Fig. 6D). Instead, SFP in lower dose and prolonged treatment reduced the NAG activity only in the group without tumor (Basal). MPO enzyme activity was not altered by any treatments (Fig. 6E).

In addition, those prolonged treatments did not result in leucocyte alterations, but an increase in platelets count in all groups was observed, when compared to Veh+Veh (Table S3). From the plasmatic parameters, glucose levels increased in all groups treated with SFP 6 mg/kg, but no changes in ALT and AST levels in these animals were observed (Table S2).

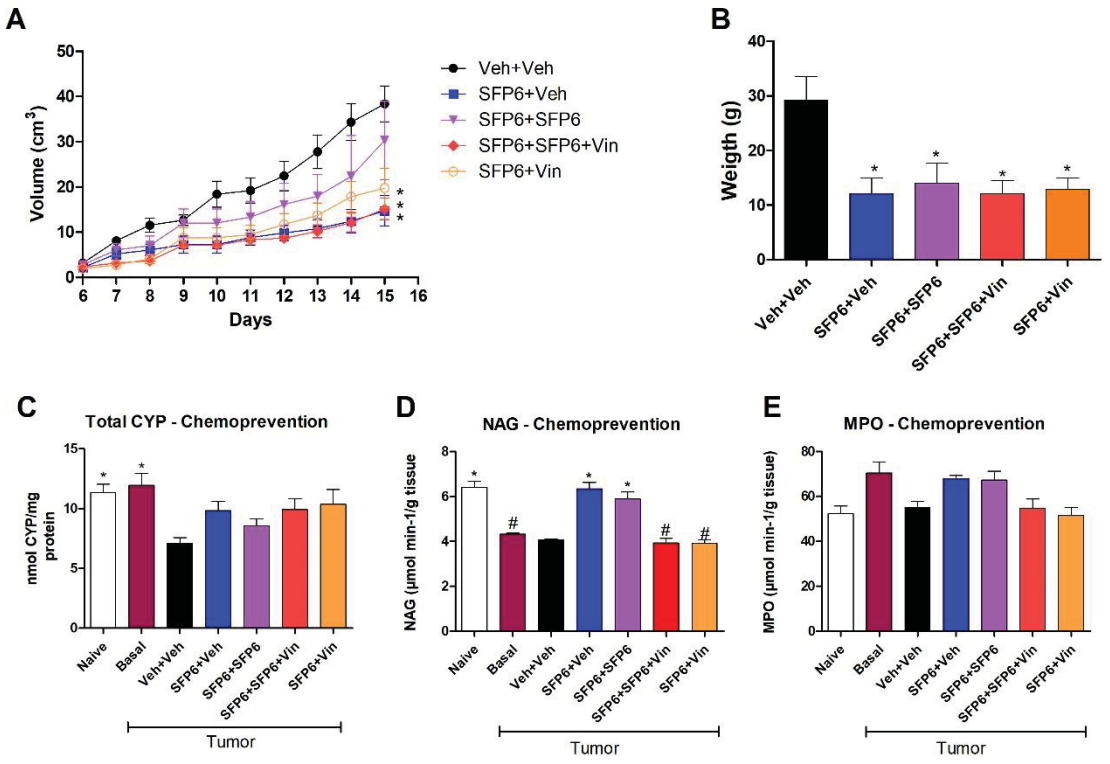

Figure 6 - Chemoprevention effects of treatments on Walker-256 tumor. Tumor volume (A) and weight (B) in Walker-256 tumor bearing-rats submitted to chemopreventive solid tumor protocol. Total CYP (C), N-acetylglucosaminidase (NAG) (D) and Myeloperoxidase (MPO) (E) in liver of rats without tumor (Naive), treated with SFP 6 mg/kg + SFP 6 mg/kg (Basal); and Walker-256 tumor bearing-rats treated with Vehicle (Veh+Veh), SFP 6 mg/kg + Vehicle (SFP6+Veh), SFP 6 mg/kg + SFP 6 mg/kg (SFP6+SFP6), SFP 6 mg/kg + SFP6 mg/kg + Vincristine 0.5 mg/kg (SFP6+SFP6+Vin), and SFP 6 mg/kg + Vincristine 0.5 mg/kg (SFP6+Vin), during pre-treatment (14 days) + treatment after tumor inoculation (14 days) in chemoprevention protocol. The values are expressed as the mean ± S.E.M. of 6-8 rats. The one-way ANOVA followed by Tukey's multiple comparisons test was used to statistical analysis. Symbol: * $p < 0.05$ as compared to the Vehicle group; # $p < 0.05$ as compared to the naive group.

3.4.3.2 Total CYP levels in intestine and *in vitro* SFP digestion

In view of the absence of alteration in hepatic CYP by SFP applied in long treatment (chemoprevention solid tumor protocol; Fig. 6C), we verified if the intestine CYP enzymes could be altered. The results indicated that total CYP levels in intestine were not modified by the treatments or by the Walker-256 tumor presence (Fig. 7). We observed that the intestinal CYP activity (0.4-1.8 nmol CYP/mg protein) was ~10-folder lower than the hepatic CYP (4.8-14.7 nmol CYP/mg protein; Fig. 6C) in rats submitted to the same chemopreventive protocol. Interestingly, the SFP structure was slightly modified in the *in vitro* gastrointestinal digestion, resulting in 1.7 mg of product after ethanol precipitation, with no significant changes in composition

(Table 1). This result indicates that SFP was probably not digested in stomach and intestine after oral administration to the rats.

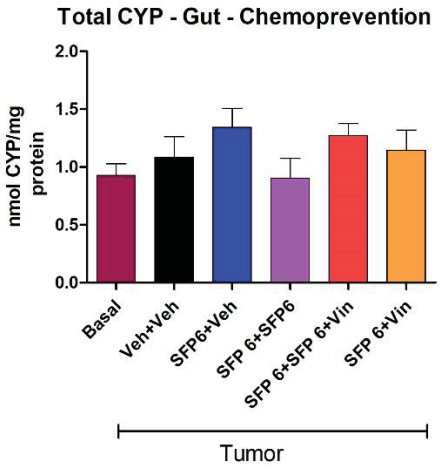

Figure 7 –– Total CYP levels in intestine. Total CYP in gut of rats without tumor, treated with SFP 6 mg/kg + SFP 6 mg/kg (Basal), and Walker-256 tumor bearing-rats treated with Vehicle (Veh+Veh), SFP 6 mg/kg + Vehicle (SFP6+Veh), SFP 6 mg/kg + SFP 6 mg/kg (SFP6+SFP6), SFP 6 mg/kg + SFP6 mg/kg + Vincristine 0.5 mg/kg (SFP6+SFP6+Vin) and SFP 6 mg/kg + Vincristine 0.5 mg/kg (SFP6+Vin), during pre-treatment (14 days) + treatment after tumor inoculation (14 days) in chemoprevention protocol. The values are expressed with the mean ± S.E.M. of 6-8 rats. The one-way ANOVA followed by Tukey's multiple comparisons test was used to statistical analyze.

Table 1 – Carbohydrates composition of SFP before and after the *in vitro* digestion system.

Polysaccharide	Recovery (mg)	Mannan (δ)	Dextrin (δ)	RGI (δ)	AGII (δ)
SFP		12	13	22	54
SFP_digested	1.7	16	12	19	53

AGII: type II arabinogalactan; RGI type I rhamnogalacturonans.

3.5 DISCUSSION

The soluble polysaccharide fraction extracted from red wine showed antitumor effect in solid tumors, corroborating previous data (8), as it relevantly reduced Walker-256 solid tumor growth, an effect similar to that of vincristine. However, in the liquid tumor protocol none of the treatments showed reduction in tumor cell viability and in ascitic fluid volume. It is important to mention that ascitic Walker-256 tumor is an aggressive tumor and animals can die within a few days (4-6 days), making it more difficult for drugs to exert an effective antineoplastic effect (18).

The absence of antitumor effect on ascitic tumor did not prevent the treatments from acting on hepatic CYP and inflammatory parameters. SFP promotes an important immune modulation by increasing TNF-α in tumor cells (8). TNF-α has been described as an inhibitor of the enzymatic activity of CYP enzymes, along with other pro-inflammatory cytokines, such as IL-1 and IL-6 (19,20); environmental and genetic factors; diseases; and substances such as herbicides, alcohol, and medicinal plants (21). In addition, TNF-α is involved in the intense inflammatory process caused by different types of cancer, such as Walker-256 tumor. This tumor model is also associated with a reduced activity of CYP enzymes in the liver, and was chosen to study the effects of SFP, alone and in combination with chemotherapeutics, on these enzymes. Interestingly, SFP reduced the total level of liver CYP enzymes in animals with liquid tumor, solid tumor, and no tumor (Basal group). This data indicates that hepatic CYP enzymes can be inhibited by SFP under physiological conditions, however which mechanisms are responsible for this, directly or indirectly, are not known. In the vehicle-treated tumor group, there was a reduction in hepatic levels of total CYP in both the solid tumor and liquid tumor protocols (Fig. 4), indicating an effect of the tumor itself on the enzymes. These data are consistent with those reported in the literature, in which there was decreased metabolic activity of CYP enzymes against Walker-256 tumor in rat liver (9,22). Vincristine, herein used as a positive control, is an inhibitor of the CYP3A4 enzyme, thus its effects in reducing total CYP levels validate our experimental design (23). However, the presence of the tumor or the association with the drugs in the VS group did not result in potentiation of the effects upon the enzyme activity, since there was no reduction of CYP levels beyond the reductions already found in each groups. Because CYP enzymes are

abundantly found in liver tissue, inhibition of one or more CYP enzymes can be surpassed by the activity of another CYP.

It is important to emphasize that the observed effects on CYP enzymes did not result in liver injury, since plasmatic ALT and AST levels (Table S2) were not altered in the tumor-bearing groups. These enzymes are physiologically present inside hepatocytes, but are important markers of cellular injury when found elevated in plasma. AST can also be altered in cases of extrahepatic injury, such as in skeletal and cardiac muscle, for example. For this reason, this enzyme is usually increased in animals with Walker-256 tumor (8,24), regardless of the treatments, as demonstrated in our results.

Additionally, SFP (Basal and SFP groups), Walker-256 tumor (Veh group), and vincristine modulated the immune-inflammatory system by increasing NAG enzyme activity and TNF-α levels in the liver of rats from the solid tumor protocol, in which total CYP was reduced. Macrophages probably increased TNF-α secretion, and this cytokine may be responsible for decreasing the levels of CYP enzymes in those groups. These results corroborate with Morgan et al. (25) and Anderson et al. (26), who evidenced that pro-inflammatory cytokines, such as TNF-α, are the main mediators of multiple CYPs regulation in the presence of inflammatory process, decreasing the activity of these enzymes. However, there was no increase in TNF-α for the liquid tumor protocol groups, despite an increase in NAG activity for the Vin and SFP groups, and MPO for the Vin group, when compared to Veh and Naive. This indicates that other cytokines may act at early stages of Walker-256 ascitic tumor, while TNF-α should be involved with later stages of solid tumor development, in which the tumor microenvironment is structured. Additionally, in liquid protocol the animals show accumulation of hemorrhagic ascitic fluid in the peritoneal cavity, probably resulting in intense recruitment of pro-inflammatory cells and release of other cytokines.

From these results, we sought to identify the specificity of CYP enzymes affected by the treatments, through mRNA expression analysis of CYP3A9, CYP2E1, CYP2D4 and CYP1A1 enzymes. CYP3A4 is the most important CYP in adult liver and CYP3A9 is its analog in rats (17,27). This enzyme was inhibited by SFP treatment in healthy rats, which did not occur in tumor-bearing animals that received SFP. Additionally, the inhibitory effect observed in Vin and VS groups was expected, since vincristine is an inhibitor of CYP3A4 (23). However, the association in VS group

did not result in drug interactions, since there was no increase in the inhibitory effect of vincristine, nor the blockade of this effect with respect to *Cyp3a9*. Importantly, the combined role of drugs and/or herbal substances could promote adverse effects, increase the efficacy of drugs, or even decrease their toxicity (28). Thus, the association between SFP and vincristine could be considered safe regarding the interactions on CYP3A4/ CYP3A9.

Considering that SFP is a compound present in the cabernet franc red wine, another enzyme evaluated was CYP2E1, involved in the metabolism of ethanol (29), which is considered an inducer of this enzyme (30). Speculatively, the inhibitory effect observed in Fig. 4B for the Basal and SFP group could modify the effects of ethanol on CYP2E1, resulting in decreased expression of this enzyme and impaired ethanol metabolism, increasing its toxic effects. However, it is not known the effect of the association of SFP and ethanol on CYPs, because the SFP preparation used in this work is completely ethanol-free. In addition, the mRNA and protein levels of CYP2E1 are increased in many cancer subtypes, such as liver hepatocellular carcinoma (31–33), breast cancer (34) and lung cancer (35), indicating a poor prognosis to these patients. Consequently, the CYP2E1 inhibition could be an additional anticancer effect to these patients. SFP, Vin and VS groups demonstrate inhibitory effect on *Cyp2e1* gene, however no synergism was observed between the association of SFP and vincristine.

CYP2D4 is a rat analog of the human CYP2D6 (36). These enzymes are responsible for the metabolism of several drugs of clinical interest and used by cancer patients, such as antidepressants (37), beta-blockers (38), codeine (39), tramadol (40) and tamoxifen (41). The importance of the expression of CYP2D6 in breast tumors is still controversial. High expression of CYP2D6 has previously been associated with increased mortality in patients with different types of breast cancer and in peri-menopausal patients, while low expression would be linked to increased mortality in postmenopausal patients. It is likely that high expression is not directly linked to enzyme functionality, because even patients with slow metabolism genotypes are treated with tamoxifen, an antiestrogen drug used for hormone receptor positive breast cancer, which is metabolized by CYP2D6. On the other hand, Hertz et al. (42) demonstrated that genotypes of CYP2D6 are not associated with mortality in breast cancer patients, specifically, those treated with tamoxifen. In

the present study the homolog CYP2D4 was evaluated and showed no differences among the treatments.

Another essential enzyme for our study is CYP1A1, as this enzyme is related to the detoxification of compounds with carcinogenic properties and contributes to cancer progression (43). The expression of CYP1A1, CYP2A6, CYP2E1 and several CYP3A is increased in lung adenocarcinoma, and this expression is more frequent in women, suggesting a higher risk of women developing this type of adenocarcinoma (44). Furthermore, CYP1A1 polymorphisms are predictors of susceptibility to lung cancer (45), while increased expression of the CYP1 family indicates carcinogenesis from xenobiotic exposure in cancers of endometrium and ovary (46). Consequently, the inductive effect of CYP1A1 observed in the Veh group in Walker-256-bearing rats and in the DMEM group in HepG2 cells is consistent with the literature. Thus, considering the effect of CYP1A on cancer development, it is important to note that both SFP in tumor-bearing animals and SFP, vincristine, and cisplatin in HepG2 cells promoted inhibition of CYP1A1 and CYP1A2 expression, respectively, resulting in basal expression levels. However, CYP1A inhibition is unrelated to the antitumor effect of SFP, as no cytotoxic effect of SFP in HepG2 cultures was observed. CYP1A2 inhibition may be a mechanism that alters the cell sensitivity to chemotherapy, but this hypothesis still needs investigation. In contrast, in absence of an in vitro immune system, the enzymatic inhibition found in HepG2 cells may be triggered directly by the substances used, or by stimulating the synthesis of proinflammatory cytokines by HepG2 cells (47).

The effects of SFP in the chemoprevention protocol against Walker-256 solid tumor were also evaluated. A low dose of SFP (6 mg/kg) in prolonged therapy induced a significant antitumor effect. However, the low dose of SFP did not promote modulation in the immune system, and perhaps this effect was the cause of no reduction in hepatic CYP levels. These data indicate that high doses of SFP are necessary to induce the immune system and the production of pro-inflammatory cytokines, which are responsible for the inhibitory effects on CYPs. Thus, the antitumor effect of SFP is not only dependent of immune regulation, but of other mechanisms that deserve future investigation. Additionally, the intestine of animals submitted to the chemopreventive protocol was analyzed to identify a possible modulation of treatment on intestinal CYPs. Although chronic treatment with different polysaccharides may result in modulation in the intestinal microbiome (48) and in the

gene expression of cellular transporters (49) and metabolism enzymes (50), SFP did not promote alteration in the levels of total intestinal CYP, at least during the 28-day treatment.

It is very likely that SFP also did not change its composition when passing through the digestive system of rats, based on the slight change in its chemical structure after the *in vitro* digestion assay and NMR evaluation. A similar effect has already been observed in studies with the polysaccharide rhamnogalacturonan (RGal), for which pH changes caused by the simulated digestive fluids probably caused de-esterification of RGal. Despite this, the other $^{13}C/^{1}H$ correlations did not change after the treatment, indicating that RGal still remained as a polysaccharide (14). Although it is not known whether SFP structure can directly inhibit CYP activity, the permanence of SFP structure in the cell-free digestion system and unchanged intestinal CYP activity reinforce the hypothesis that SFP modulates CYPs mediated by cytokines and immune-inflammatory system, rather than by direct action.

In conclusion, SPF administered by oral via reaches intactly the intestine, prevents tumor growth, and promotes modulation of the immune system and CYP levels in the liver. These effects, however, were observed with different dosages. The antineoplastic effect of SFP against the solid Walker-256 tumor occurs over a wide dose range (6 and 60 mg/kg), while a higher dose is required to induce immunomodulation and inhibition of hepatic CYPs. SFP treatment decreased the gene expression of some CYPs, especially from the CYP1A family, both in the liver of tumor-bearing rats and in cultured HepG2 cells. This is a relevant effect of SFP, since CYPs from the family 1A are related with carcinogenesis, which reinforces the potential of SFP as an antineoplastic compound.

3.6 ACKNOWLEDGMENT

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3.7 REFERENCES

1. Manikandan P, Nagini S. Cytochrome P450 Structure, Function and Clinical Significance: A Review. Curr Drug Targets. 2017;

2. Tornio A, Backman JT. Cytochrome P450 in Pharmacogenetics: An Update. In 2018. p. 3–32. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1054358918300267

3. Zanger UM, Schwab M. Cytochrome P450 enzymes in drug metabolism: Regulation of gene expression, enzyme activities, and impact of genetic variation. Pharmacol Ther [Internet]. 2013 Apr;138(1):103–41. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0163725813000065

4. Shah P, Guo T, Moore DD, Ghose R. Role of constitutive androstane receptor in toll-like receptor-mediated regulation of gene expression of hepatic drug-metabolizing enzymes and transporters. Drug Metab Dispos. 2014;42(1):172–81.

5. Christensen H, Hermann M. Immunological Response as a Source to Variability in Drug Metabolism and Transport. Front Pharmacol [Internet]. 2012;3:8. Available from: http://journal.frontiersin.org/article/10.3389/fphar.2012.00008/abstract

6. Agundez J a G. Cytochrome P450 gene polymorphism and cancer. Curr Drug Metab [Internet]. 2004;5(3):211–24. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15180491

7. Shi J, Geng MY, Liu CX. Comparative studies of the effects of two novel sugar drug candidates on the CYP 1A2 and CYP 2E1 enzymes in different sexed rats using a “cocktail” approach. Molecules. 2004;9(11):978–87.

8. Stipp MC, Bezerra I de L, Corso CR, dos Reis Livero FA, Lomba LA, Caillot ARC, et al. Necroptosis mediates the antineoplastic effects of the soluble fraction of polysaccharide from red wine in Walker-256 tumor-bearing rats. Carbohydr Polym [Internet]. 2017;160:123–33. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0144861716314187

9. Olson JW, Weiner M. The relationship of the cyclic nucleotide system to inhibition of hepatic drug metabolism in Walker 256 carcinoma-bearing rats. Res Commun Chem Pathol Pharmacol [Internet]. 1980;30(1):71–89. Available from: http://www.ncbi.nlm.nih.gov/pubmed/6254121

10. Strober W. Trypan Blue Exclusion Test of Cell Viability. Curr Protoc Immunol [Internet]. 2015 Nov 2;111(1). Available from: https://onlinelibrary.wiley.com/doi/10.1002/0471142735.ima03bs111

11. Matsubara T, Koike M, Touchi A, Tochino Y, Sugeno K. Quantitative determination of cytochrome P-450 in rat liver homogenate. Anal Biochem. 1976;75(2):596–603.

12. Bradley PP, Priebat D a, Christensen RD, Rothstein G. Measurement of cutaneous inflammation: estimation of neutrophil content with an enzyme marker. Vol. 78, The Journal of investigative dermatology. 1982. p. 206–9.

13. Sánchez T, Moreno JJ. Role of prostaglandin H synthase isoforms in murine ear edema induced by phorbol ester application on skin. Prostaglandins Other Lipid Mediat. 1999;57:119–31.

14. Maria-Ferreira D, Nascimento AM, Cipriani TR, Santana-Filho AP, Watanabe P da S, Sant´Ana D de MG, et al. Rhamnogalacturonan, a chemically-defined polysaccharide, improves intestinal barrier function in DSS-induced colitis in mice and human Caco-2 cells. Sci Rep [Internet]. 2018 Dec 16;8(1):12261. Available from: http://www.nature.com/articles/s41598-018-30526-2

15. Bezerra I de L, Caillot ARC, Palhares LCGF, Santana-Filho AP, Chavante SF, Sassaki GL. Structural characterization of polysaccharides from Cabernet Franc, Cabernet Sauvignon and Sauvignon Blanc wines: Anti-inflammatory activity in LPS stimulated RAW 264.7 cells. Carbohydr Polym [Internet]. 2018 Apr;186:91–9. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0144861717314996

16. Bezerra I de L, Caillot ARC, Oliveira AF de, Santana-Filho AP, Sassaki GL. Cabernet Sauvignon wine polysaccharides attenuate sepsis inflammation and lethality in mice. Carbohydr Polym [Internet]. 2019 Apr;210:254–63. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0144861719300256

17. Kuang ZM, Huang ZJ, Li Y, Yang GP, Liu ML, Yuan H. Revealing the contribution of Cytochrome P450 to salt-sensitive hypertension using DNA microarray. Eur Rev Med Pharmacol Sci. 2013;

18. Acco, A., Bastos-Pereira, A.L. & Dreifuss AA. Characteristics and Applications of the Walker-256 Rat Tumour. . SG Pandalai; Daniel Pouliquen (Org) rat cancer Res a crucial tool all Asp Transl Stud 1 ed Trivandrum Res Signpost/ Transw Res Network,. 2012;

19. Aitken AE, Morgan ET. Gene-specific effects of inflammatory cytokines on cytochrome P450 2C, 2B6 and 3A4 mRNA levels in human hepatocytes. Drug Metab Dispos. 2007;35(9):1687–93.

20. Aitken AE, Richardson TA, Morgan ET. Regulation of Drug-Metabolizing Enzymes and Transporters in Inflammation. Annu Rev Pharmacol Toxicol [Internet]. 2006 Feb;46(1):123–49. Available from:

http://www.annualreviews.org/doi/10.1146/annurev.pharmtox.46.120604.141059

21. Stavropoulou E, Pircalabioru GG, Bezirtzoglou E. The Role of Cytochromes P450 in Infection. Front Immunol [Internet]. 2018;9(January):1–7. Available from: http://journal.frontiersin.org/article/10.3389/fimmu.2018.00089/full

22. RAW I. Effect of Extra-Hepatic Walker Sarcoma 256 on the synthesis and degradation of liver cytochromes P-450 and b5. Brazilian J Med Biol Res. 1983;16:291–5.

23. Harnicar S, Adel N, Jurcic J. Modification of vincristine dosing during concomitant azole therapy in adult acute lymphoblastic leukemia patients. J Oncol Pharm Pract. 2009;

24. ZAMIN Jr. I, MATTOS AA de, PERIN C, RAMOS GZ. A importância do índice AST/ALT no diagnóstico da esteatohepatite não-alcoólica. Arq Gastroenterol. 2002;

25. Morgan ET. Impact of infectious and inflammatory disease on cytochrome P450-mediated drug metabolism and pharmacokinetics. Clinical Pharmacology and Therapeutics. 2009.

26. Anderson GD, Peterson TC, Vonder Haar C, Farin FM, Bammler TK, MacDonald JW, et al. Effect of Traumatic Brain Injury, Erythropoietin, and Anakinra on Hepatic Metabolizing Enzymes and Transporters in an Experimental Rat Model. AAPS J. 2015;

27. Xu RA, Wen J, Tang P, Wang C, Xie S, Zhang BW, et al. Functional Characterization of 22 CYP3A4 Protein Variants to Metabolize Ibrutinib In Vitro. Basic Clin Pharmacol Toxicol. 2018;

28. Haslan H, Suhaimi FH, Das S. Herbal supplements and hepatotoxicity: A short review. Natural Product Communications. 2015.

29. Heit C, Dong H, Chen Y, Thompson DC, Deitrich RA, Vasiliou VK. The role of CYP2E1 in alcohol metabolism and sensitivity in the central nervous system. Subcell Biochem. 2013;

30. Peter Guengerich F, Avadhani NG. Roles of Cytochrome P450 in Metabolism of Ethanol and Carcinogens. Adv Exp Med Biol [Internet]. 2018;1032:15–35. Available from: http://www.ncbi.nlm.nih.gov/pubmed/30362088

31. Gao J, Zhou J, He X-P, Zhang Y-F, Gao N, Tian X, et al. Changes in cytochrome P450s-mediated drug clearance in patients with hepatocellular carcinoma in vitro and in vivo : a bottom-up approach. Oncotarget [Internet]. 2016

May 10;7(19):28612–23. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27086920%0Ahttp://www.ncbi.nlm.nih.gov/pubmed/27086920%0Ahttp://www.ncbi.nlm.nih.gov/pubmed/27086920

32. Tsunedomi R, Iizuka N, Hamamoto Y, Uchimura S, Miyamoto T, Tamesa T, et al. Patterns of expression of cytochrome P450 genes in progression of hepatitis C virus-associated hepatocellular carcinoma. Int J Oncol [Internet]. 2005;27(3):661–7. Available from: http://www.ncbi.nlm.nih.gov/sites/entrez?Db=pubmed&Cmd=Retrieve&list_uids=16077914&dopt=abstractplus%5Cnpapers://5aecfcca-9729-4def-92fe-c46e5cd7cc81/Paper/p16893

33. Yan T, Lu L, Xie C, Chen J, Peng X, Zhu L, et al. Severely Impaired and Dysregulated Cytochrome P450 Expression and Activities in Hepatocellular Carcinoma: Implications for Personalized Treatment in Patients. Mol Cancer Ther [Internet]. 2015;14(12):2874–86. Available from: http://mct.aacrjournals.org/cgi/doi/10.1158/1535-7163.MCT-15-0274

34. Vaclavikova R, Hubackova M, Stribrna-Sarmanova J, Kodet R, Mrhalova M, Novotny J, et al. RNA expression of cytochrome P450 in breast cancer patients. Anticancer Res [Internet]. 2007;27(6C):4443–50. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18214058

35. Oyama T. Cytochrome P450 expression (CYP) in non-small cell lung cancer. Front Biosci [Internet]. 2007;12(1):2299. Available from: http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=med5&AN=17127240%5Cnhttp://nt2yt7px7u.search.serialssolutions.com/?sid=OVID:Ovid+MEDLINE%28R%29+%3C2004+to+2007%3E&genre=article&id=pmid:17127240&id=doi:&issn=1093-9946&volume=12&issue=&

36. Hiroi T, Chow T, Imaoka S, Funae Y. Catalytic specificity of CYPD2D isoforms in rat and human. Drug Metab Dispos. 2002;

37. Bérard A, Gaedigk A, Sheehy O, Chambers C, Roth M, Bozzo P, et al. Association between CYP2D6 genotypes and the risk of antidepressant discontinuation, dosage modification and the occurrence of maternal depression during pregnancy. Front Pharmacol. 2017;

38. Luzum JA, Sweet KM, Binkley PF, Schmidlen TJ, Jarvis JP, Christman MF, et al. CYP2D6 Genetic Variation and Beta-Blocker Maintenance Dose in Patients with Heart Failure. Pharm Res. 2017;

39. Crews KR, Gaedigk A, Dunnenberger HM, Leeder JS, Klein TE, Caudle KE, et al. Clinical pharmacogenetics implementation consortium guidelines for cytochrome P450 2D6 genotype and codeine therapy: 2014 Update. Clin Pharmacol Ther. 2014;

40. Frost DA, Soric MM, Kaiser R, Neugebauer RE. Efficacy of Tramadol for Pain Management in Patients Receiving Strong Cytochrome P450 2D6 Inhibitors. Pharmacotherapy. 2019;

41. Brooks JD, Comen EA, Reiner AS, Orlow I, Leong SF, Liang X, et al. CYP2D6 phenotype, tamoxifen, and risk of contralateral breast cancer in the WECARE Study. Breast Cancer Res. 2018;

42. Hertz DL, Kidwell KM, Hilsenbeck SG, Oesterreich S, Osborne CK, Philips S, et al. CYP2D6 genotype is not associated with survival in breast cancer patients treated with tamoxifen: results from a population-based study. Breast Cancer Res Treat. 2017;

43. Androutsopoulos VP, Tsatsakis AM, Spandidos D a. Cytochrome P450 CYP1A1: wider roles in cancer progression and prevention. BMC Cancer [Internet]. 2009;9(1):187. Available from: http://bmccancer.biomedcentral.com/articles/10.1186/1471-2407-9-187

44. Oyama T, Sugio K, Isse T, Matsumoto A, Nose N, Uramoto H, et al. Expression of cytochrome P450 in non-small cell lung cancer. Front Biosci [Internet]. 2008 May 1;13:5787–93. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18508622

45. Song N. CYP 1A1 polymorphism and risk of lung cancer in relation to tobacco smoking: a case-control study in China. Carcinogenesis [Internet]. 2001 Jan 1;22(1):11–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11159735

46. Go RE, Hwang KA, Choi KC. Cytochrome P450 1 family and cancers. J Steroid Biochem Mol Biol. 2015;147:24–30.

47. Silva CS da. Apoptose induzida por palmitato em células hepg2 depende da produção de tnf- α lfa. 2012;1–77.

48. Porter NT, Martens EC. The Critical Roles of Polysaccharides in Gut Microbial Ecology and Physiology. Annual Review of Microbiology. 2017.

49. Zhang T, Yang Y, Liang Y, Jiao X, Zhao C. Beneficial effect of intestinal fermentation of natural polysaccharides. Nutrients. 2018.

50. Li WF, Feng J, Xu ZR, Yang CM. Effects of non-starch polysaccharides

enzymes on pancreatic and small intestinal digestive enzyme activities in piglet fed diets containing high amounts of barley. World J Gastroenterol. 2004;

3.8 SUPPLEMENTARY MATERIAL

Supplementary Table S1 – Sequences of rat and human primers used in qPCR assays.

Gene	Primer Sequence (5'-3')
CYP3A9-rat (F)	TTGCCTTTCTTGGGGACGAT
CYP3A9-rat (R)	ACTGGACCAAAGTTCCGTCG
CYP2E1-rat (F)	TTCACCAAGTTGGCAAAGCG
CYP2E1-rat (R)	AGGCTGGCCTTTGGTCTTTT
CYP2D4-rat (F)	CTCCAGACTTCTCGACTTGGTT
CYP2D4-rat (R)	GGGTTTCTTTGGAAACACCTC
CYP1A1-rat (F)	TGAGACAGTATTGTGTAGTCCAAGT
CYP1A1-rat (R)	CACTTGGTAGGGTGGTAAAAGC
GAPDH-rat (F)	AAGGACCCCTTCATTGAC
GAPDH-rat (R)	TCCACGACATACTCAC
CYP1A2-human (F)	GACATCTTTGGAGCAGGATTTGA
CYP1A2-human (R)	CTTCCTCTGTATCTCAGGCTTGGT
CYP2B6-human (F)	ACATCGCCCTCCAGAGCTT
CYP2B6-human (R)	GTCGGAAAATCTCTGAATTCTCATAGA
GAPDH-human (F)	CATGAGAAGTATGACAACAGCC
GAPDH-human (R)	AGTCCTTCCACGATACCAAAG

Supplementary Table S2 - Plasmatic parameters of Walker-256 bearing-rats treated according to the *a)* Solid tumor and *c)* Chemopreventive solid tumor protocols.

	Solid Tumor Protocol					**Chemopreventive Solid Tumor Protocol**				
	Veh	**SFP**	**Vin**	**VS**	**Basal**	**Veh + Veh**	**SFP6 + Veh**	**SFP6 + SFP6**	**SFP6 + SFP6 + Vin**	**SFP6 + Vin**
Glucose	62.9	62.0	78.2*	74.0*	166.6#	88.1	113.9#	118.8#	134.0#	121.5#
ALT	57.7	41.7	65.6	63.1	51.5	51.8	45.9	42.0	51.5	51.7
AST	221.4	158.8*	176.7*	158.3*	83.4#	180.5	140.8	165.3	169.4	204.5

Solid tumor protocol was composed by animals with tumor, treated with vehicle (Veh), SFP 60 mg/kg (SFP), Vincristine 0.5 mg/kg (Vin) and SFP 60 mg/kg + Vincristine 0.5 mg/kg (VS), during 14 days. Chemoprevention solid tumor protocol was composed by animals without tumor, treated with SFP 6 + 6 mg/kg (Basal), and Walker-256 tumor bearing-rats treated with Vehicle (Veh+Veh), SFP 6 mg/kg + Vehicle (SFP6+Veh), SFP 6 mg/kg + SFP 6 mg/kg (SFP6+SFP6), SFP 6 mg/kg + SFP6 mg/kg + Vincristine 0.5 mg/kg (SFP6+SFP6+Vin), and SFP 6 mg/kg + Vincristine 0.5 mg/kg (SFP6+Vin), during

pre-treatment (14 days) + treatment after tumor inoculation (14 days) in chemoprevention protocol. The values are expressed with the mean ± S.E.M. of 6-8 rats. The one-way ANOVA followed by Tukey's multiple comparisons test was used to statistical analyze. Symbols: $p < 0.05$, # when compared to Veh+Veh group; *$p < 0.05$ when compared to Veh group.

Supplementary Table S3 - Hematological parameters evaluated in Walker-256 bearing-rats treated according to the *a)* Solid tumor and *b)* Chemopreventive solid tumor protocols.

	Solid Tumor Protocol					**Chemopreventive Solid Tumor Protocol**				
	Veh	**SFP**	**Vin**	**VS**	**Basal**	**Veh + Veh**	**SFP6 + Veh**	**SFP6 + SFP6**	**SFP6 + SFP6 + Vin**	**SFP6 + Vin**
Erythrocytes (x10^6/µl)	7.7	8.9	6.6	6.5	8.2	8.0	8.0	7.8	7.4	7.0
Hematocrit (%)	49.3	--	42.3	42.3	51.9	51.2	52.6	50.7	46.9	46.0
Hemoglobin (g/dL)	15.5	14.3	13.5	13.0	16.6	16.2	16.3	15.9	14.8	14.5
VCM (fL)	64.3	50	63.8	65.5	63.2	64.0	65.5	65.5	63.3	65.0
HCM (pg)	20.1	15.9	20.4	20.1	20.3	20.4	20.2	20.4	20.0	20.4
CHCM (%)	31.2	32.0	31.9	30.8	32.1	31.8	30.9	31.2	31.7	31.3
Platelets (x10^3/µl)	481	366	882*	917*	993#	647	980#	670#	1.087#	1.148#
Leukocytes (/µl)	17057.1	13000	15614.3	17566.7	13057.1	17135.7	15971.4	18728.6	13714.3	15557.1
Segmented Neutrophils (/µl)	6575.7	3800	7623.3	9545.0	2414.0#	7319.4	5014.7	8459.1	6429.0	7907.4
Bastonetes (/µl)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Lymphocytes (/µl)	10062.6	8850	7709.9*	7763.3*	10449.3	9401.4	10538.6	9739.6	7013.1	7279.9
Monocytes (/µl)	390.3	366	281.1	258.3	177.3	390.0	319.6	501.6	272.1	350.6
Eosinophils (/µl)	48.6	0.0	0.0	0.0	16.0	35.0	47.4	28.3	0.0	0.0

Solid tumor protocol was composed by animals with tumor, treated with vehicle (Veh), SFP 60 mg/kg (SFP), Vincristine 0.5 mg/kg (Vin) and SFP 60 mg/kg + Vincristine 0.5 mg/kg (VS), during 14 days. Chemoprevention solid tumor protocol was composed by animals without tumor, treated with SFP 6 + 6 mg/kg (Basal), and Walker-256 tumor bearing-rats treated with Vehicle (Veh+Veh), SFP 6 mg/kg + Vehicle (SFP6+Veh), SFP 6 mg/kg + SFP 6 mg/kg (SFP6+SFP6), SFP 6 mg/kg + SFP6 mg/kg + Vincristine 0.5 mg/kg (SFP6+SFP6+Vin), and SFP 6 mg/kg + Vincristine 0.5 mg/kg (SFP6+Vin), during pre-treatment (14 days) + treatment after tumor inoculation (14 days) in chemoprevention protocol. The values are expressed with the mean ± S.E.M. of 6-8 rats. The one-way ANOVA followed by Tukey's multiple comparisons test was used to statistical analyze. Symbols: $p < 0.05$, # when compared to Veh+Veh group; *$p < 0.05$ when compared to Veh group.

4 CONSIDERAÇÕES FINAIS

Os efeitos da fração solúvel do polissacarídeo extraído do vinho tinto sobre a tríade citocromo P450-inflamação-câncer são evidenciados neste trabalho. Isso ocorre por meio do aumento de NAG e TNF-α no tecido hepático tanto de animais portadores de tumor sólido, quanto de animais saudáveis, tratados com a dose de SFP 60 mg/kg por 14 dias, associada ou não ao quimioterápico vincristina, um inibidor da CYP3A4. Este efeito aparentemente é dose-dependente e tempo-dependente, visto que os resultados encontrados com a dose de 60 mg/kg em 5 e 14 dias, ou com a dose de 6 mg/kg por 28 dias são diferentes quanto à modulação imunológica. De modo específico, a menor dose (6 mg/kg, via oral) por um período prolongado (28 dias) não causa modulação imunológica sanguínea e hepática. No entanto, a dose de 60 mg/kg por via oral resulta em efeitos imunomoduladores, que promovem a inibição das CYPs, o que não ocorre com a dose de 6 mg/kg. Associado a esses achados, apesar da ausência de efeito imunomodulador, observou-se que a dose de 6 mg/kg possui um relevante efeito antitumoral, que possivelmente está associado a mecanismos de ação independentes da via inflamatória, que ainda não foram investigados.

A diminuição nos níveis totais de CYP, e especificamente, da expressão das enzimas CYP1A1, CYP2E1 e CYP3A9 demonstram a necessidade de cautela na prescrição de fármacos, caso fossem associados à SFP, a fim de evitar interações medicamentosas. Mas é importante ressaltar que o efeito antitumoral da dose de 60 mg/kg da SFP é extremamente promissor, pois além dos efeitos descritos anteriormente, também houve inibição das *Cyp1a1* e *Cyp2e1* in vivo e *CYP1A2* in vitro em células hepáticas. Esses dados são relevantes, uma vez que indicam que a SFP pode estimular a produção de citocinas pró-inflamatórias pelas células HepG2, ou ainda, atuar diretamente sobre as células hepáticas, levando à inibição da *CYP1A2*. Essas enzimas estão diretamente ligadas à carcinogênese, e o aumento da sua expressão tem sido relacionada com o pior prognóstico de pacientes portadores de diferentes tipos de câncer. Desta forma, a sua inibição pode ser benéfica no tratamento desses pacientes, o que evidencia o potencial terapêutico dos polissacarídeos aqui investigados.

Consequentemente, apesar da diversidade do papel das diferentes doses da SFP sobre a tríade citocromo P450-inflamação-câncer, fica clara a importância

dessa substância como futuro antineoplásico. Adicionalmente, é fundamental evidenciar o uso da SFP em pacientes portadores de cânceres com superexpressão da sub-família CYP1A e da CYP2E1, pois além dos efeitos antitumorais já esclarecidos da dose de 60 mg/kg, encontramos um efeito adicional, através da inibição dessas enzimas, o que por sua vez, pode ser benéfico a esse grupo de pacientes.

4.1 RECOMENDAÇÕES PARA TRABALHOS FUTUROS

Recomenda-se investigar se há o envolvimento de outras citocinas pró-inflamatórias na inibição encontrada sobre as enzimas CYP nos protocolos de tratamentos aqui estudados, assim como buscar pela possível via intracelular que leva a essa inibição. Outro ponto a ser desvendado são os efeitos da SFP na expressão proteica dessas enzimas, visto que este trabalho focou na análise de expressão do mRNA. Por fim, seria interessante investigar os mecanismos que levam à ausência de inibição de CYPs frente ao tratamento prolongado com uma dose baixa da SFP.

REFERÊNCIAS

ACCO, A.; BASTOS-PEREIRA, A. L.; DREIFUSS, A. A. Characteristics and Applications of the Walker-256 Rat Tumor. In: S.G. Pandalai; Daniel Pouliquen. (Org.). The rat in cancer research: a crucial tool for all aspects of translational studies. 1 ed. Trivandrum: Research Signpost/ Transworld Research Network, 2012.

AHMED EM, EL-MARAGHY SA, TELEB ZA, SHAHEEN AA. Pretreatment with turmeric modulates the inhibitory influence of cisplatin and paclitaxel on CYP2E1 and CYP3A1/2 in isolated rat hepatic microsomes. Chem Biol Interact. V. 220, p. 25–32, 2014. https://doi.org/10.1016/j.cbi.2014.05.007

BERNHARDT, R. Cytochromes P450 as versatile biocatalysts. J. Biotechnol. V. 124, p. 128-45, 2006. Disponível em: https://www.sciencedirect.com/science/article/abs/pii/S016816560600085X?via%3Dihub

BLOOR, R.; HAVEN, L. The Weight and Lipid Content of the Intestines in Rats AND. N. 9. 1954.

BUCHBINDER, E.L.; DESAI A. CTLA-4 and PD-1 Pathways: Similarities, Differences, and Implications of Their Inhibition. Am J Clin Oncol. v. 39, p. 98-106, 2016. doi: 10.1097/COC.0000000000000239.

CARVALHO, M. I.; SILVA-CARVALHO, R.; PIRES, I.; PRADA, J.; BIANCHINI, R.; JENSEN-JAROLIM, E.; QUEIROGA, F. L. A Comparative Approach of Tumor-Associated Inflammation in Mammary Cancer between Humans and Dogs. BioMed Research International. V. 2016, p. 1-12, 2016. Disponível em: https://www.hindawi.com/journals/bmri/2016/4917387/

DASARI S, TCHOUNWOU PB. Cisplatin in cancer therapy: molecular mechanisms of action. *Eur J Pharmacol*. 2014;740:364-378. doi:10.1016/j.ejphar.2014.07.025

DE JONG, L. M.; JISKOOT W.; SWEN, J. J.; MANSON, M. L. Distinct Effects of Inflammation on Cytochrome P450 Regulation and Drug Metabolism: Lessons from Experimental Models and a Potential Role for Pharmacogenetics. Genes (Basel). 2020;11(12):1509. doi:10.3390/genes11121509

DONATO, M.T.; TOLOSA, L.; GÓMEZ-LECHÓN, M.J. Culture and Functional Characterization of Human Hepatoma HepG2 Cells. Methods Mol Biol. v.1250, p. 77-93, 2015. doi: 10.1007/978-1-4939-2074-7_5.

EARLE, W. R. Am. J. Cancer. 24, 566, 1934.

EGBELAKIN A, FERGUSON MJ, MACGILL EA, et al. Increased risk of vincristine neurotoxicity associated with low CYP3A5 expression genotype in children with acute lymphoblastic leukemia. Pediatr Blood Cancer 56:361–367, 2011. https://doi.org/10.1002/pbc.22845

ELINAV, E.; NOWARSKI, R.; THAIS, C. A.; HU, B.; JIN, C.; FLAVELL, R. A. Inflammation-induced cancer: crosstalk between tumours, immune cells and microorganisms. Nature Reviews. Cancer. v. 13, n. 759-71, 2013. Disponível em: < https://www.nature.com/articles/nrc3611>

FRERES P, JERUSALEM G, MOONEN M. Categories of Anticancer Treatments. Anticancer Treat. Cardiotoxicity Mech. Diagnostic Ther. Interv., 2017. https://doi.org/10.1016/B978-0-12-802509-3.00002-9.

GILANI, B.; CASSAGNOL, M. Biochemistry, Cytochrome P450. 2021. Disponível em: https://www.ncbi.nlm.nih.gov/books/NBK557698/

GOLDACRE, R. J.; SYLVEN, B. No title. Brit. J. Cancer. v. 16, p. 306, 1962.

GRIVENNIKOV, S. R.; GRETEN, F. R.; KARIN, M. Immunity, Inflammation, and Cancer. Cell, v. 140, n. 6, p. 883-899, 2011. Disponível em: < https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2866629/#:~:text=Cancer%20therapy%20can%20also%20trigger,to%20immune%2Dmediated%20tumor%20eradication.>

GURUSAMY, D.; CLEVER, D.; EIL, R; RESTIFO, N.P. Novel “Elements” of Immune Suppression within the Tumor Microenvironment. Cancer Immunol Res June 1 v. 5; 6, p. 426-433, 2017. doi: 10.1158/2326-6066.CIR-17-0117

HANAHAN, D.; WEINBERG, R. A. Hallmarks of cancer: The next generation. Cell. 4;144(5):646-74. doi: 10.1016/j.cell.2011.02.013. Disponível em: < https://pubmed.ncbi.nlm.nih.gov/21376230/ >

HIAM-GALVEZ, K.J., ALLEN, B.M. & SPITZER, M.H. Systemic immunity in cancer. *Nat Rev Cancer. v.* 21, p**.** 345–359, 2021. https://doi.org/10.1038/s41568-021-00347-z

HOLOHAN, C.; SCHAEYBROECK, S. V.; LONGLEY, D. B.; JOHNSTON, P. G. Cancer drug resistance: an evolving paradigm. 13, 7140726, 2013. Disponível em: < https://www.nature.com/articles/nrc3599 >

INCA, BRASIL. Ministério da saúde. Incidência de Câncer no Brasil – Estimativa 2014. Rio de Janeiro: Instituto Nacional de Câncer José Alencar Gomes da Silva, 2011. Disponível em: http://www.inca.gov.br/estimativa/2014/estimativa-24042014.pdf.

JUNTTILA, M. R.; DE SAUVAGE, F. J. Influence of tumour micro-environment heterogeneity on therapeutic response. Nature, v. 501, n. 7467, p. 346-354, 2013. Disponível em: http://www.nature.com/doifinder/10.1038/nature12626

LI J, LI D, TIE C, et al. Cisplatin-mediated cytotoxicity through inducing CYP4A 11 expression in human renal tubular epithelial cells. J Toxicol Sci 40:895–900, 2015. https://doi.org/10.2131/jts.40.895

MANTOVANI. A.; MARCHESI, F.; MALESCI, A.; LAGHI, L.; ALLAVENA, P. Tumour-associated macrophages as treatment targets in oncology. Nature Reviews Clinical Oncology, 2017. Disponível em: < https://www.nature.com/articles/nrclinonc.2016.217>

OLSON, J. W.; WEINER, M. The relationship of the cyclic nucleotide system to inhibition of hepatic drug metabolism in Walker-256 carcinoma-bearing rats. Research communications in chemical pathology and pharmacology, v. 30, n. 1, p-71-89, 1980. Disponível em: http://www.ncbi.nlm.nih.gov/pubmed/6254121

PERROUD, A. P. A. S.; ASHIMINE, R.; DE CASTRO, G. M.; GUIMARÃES, F.; VIEIRA, K. P.; VILELLA, C. A.; CAVALCANTI, T. C. S.; DE LI, R. Cytokine gene expression in Walker-256: A comparasion of variants A (aggressive) and AR (regressive). Cytokine, v. 36, n. 3-4, p. 123-133, 2006. DOI: 10.1016/j.cyto.2006.11.004.

RAW, I. Effect of extra-hepatic Walker sarcoma 256 on the synthesis and degradation of liver cytochromes P-450 and b5. Brazilian Journal of Medical Biological Research., v. 16, p. 291-295, 1983.

ROCHA, C. R. R. Mecanismos de resistência à quimioterápicos em células tumorais, 2016.

SHAH R.R.; SMITH R.L. Inflammation-induced phenoconversion of polymorphic drug metabolizing enzymes: Hypothesis with implications for personalized medicine. Drug Metab. Dispos, v. 43, p. 400-410, 2015. doi: 10.1124/dmd.114.061093. Disponível em: https://dmd.aspetjournals.org/content/43/3/400.long

SIMPKINS, H.; LEHMAN, J. M.; MAZURKIEWICZ, J. E.; DAVIS, B. H. A morphological and phenotypic analysis of Walker 256 cells. p. 1334-1338, 1991. Disponível em: https://cancerres.aacrjournals.org/content/51/4/1334

STAVROPOULOU, E. PIRCALABIORU, G. G.; BEZIRTZOGLOU, E. The Role of Cytochromes P450 In Infection. Front Immunol. V. 31. P.89. 2018. Disponível em: https://www.frontiersin.org/articles/10.3389/fimmu.2018.00089/full

STIPP, M. C.; BEZERRA, I. L.; CORSO, C. R.; LIVERO, F. A. R.; LOMBA, L. A.; CAILLOT, A. R. C.; ZAMPRONIO, A. R.; QUEIROZ-TELLES, J. E.; KLASSEN, G.; RAMOS, E. A. S.; SASSAKI, G. L.; ACCO, A. Necroptosis mediates the antineoplastic effects of the soluble fraction of polysaccharide from red wine in Walker-256 tumor-bearing rats. Carbohydrate Polymers, v. 160, p. 123-133, 2017. Disponível em: https://linkinghub.elsevier.com/retrieve/pii/S0144-8617(16)31418-7

WANG, X.; LIN, Y. Tumor necrosis factor and cancer, buddies or foes? Acta Pharmacologica Sinica. V. 29, 1275-1288, 2008. Disponível em: https://www.nature.com/articles/aps2008156

ZANGER, U. M.; SCHWAB, M. Cytochrome P450 enzymes in drug metabolism: Regulation of gene expression, enzymes activities, and impact of genetic variation. Pharmacology and Therapeutics. V. 138, p. 103-141, 2013. https://www.sciencedirect.com/science/article/pii/S0163725813000065?via%3Dihub

PRODUÇÃO CIENTÍFICA DURANTE O DOUTORADO

1. Alves de Souza CE, Alves de Souza HM, **Stipp MC,** Corso CR, Galindo CM, Cardoso CR, Dittrich RL, de Souza Ramos EA, Klassen G, Carlos RM, Correia Cadena SMS, Acco A. Ruthenium complex exerts antineoplastic effects that are mediated by oxidative stress without inducing toxicity in Walker-256 tumor-bearing rats. Free Radic Biol Med. 2017 Sep;110:228-239. doi: 10.1016/j.freeradbiomed.2017.06.011. Epub 2017 Jun 17. PMID: 28629835.

2. Kanazawa, Luiz K.S.; Vecchia, Débora D.; Wendler, Etiéli M.; Hocayen, Palloma De A.S.; Berão, Paulo S.; De Mélo, Manuela L.; Dos Reis Lívero, Francislaine A.; Corso, Claudia Rita; **Stipp, Maria Carolina**; Acco, Alexandra; Andreatini, Roberto. Effects of acute and chronic quercetin administration on methylphenidate-induced hyperlocomotion and oxidative stress. LIFE SCIENCES JCR, v. 171, p. 1-8, 2017.

3. Adami ER, Corso CR, Turin-Oliveira NM, Galindo CM, Milani L, **Stipp MC**, do Nascimento GE, Chequin A, da Silva LM, de Andrade SF, Dittrich RL, Queiroz-Telles JE, Klassen G, Ramos EAS, Cordeiro LMC, Acco A. Antineoplastic effect of pectic polysaccharides from green sweet pepper (Capsicum annuum) on mammary tumor cells in vivo and in vitro. Carbohydr Polym. 2018 Dec 1;201:280-292. doi: 10.1016/j.carbpol.2018.08.071. Epub 2018 Aug 20. PMID: 30241820.

4. Corso CR, **Stipp MC**, Adami ER, da Silva LM, Mariott M, de Andrade SF, de Souza Ramos EA, Klassen G, Beltrame OC, Queiroz-Telles JE, de Oliveira CS, Stefanello MÉA, Acco A. Salvia lachnostachys Benth has antitumor and chemopreventive effects against solid Ehrlich carcinoma. Mol Biol Rep. 2019 Oct;46(5):4827-4841. doi: 10.1007/s11033-019-04931-3. Epub 2019 Jul 3. PMID: 31270760.

5. Milani L, Galindo CM, Turin de Oliveira NM, Corso CR, Adami ER, **Stipp MC**, Beltrame OC, Acco A. The GLP-1 analog liraglutide attenuates acute liver injury in mice. Ann Hepatol. 2019 Nov-Dec;18(6):918-928. doi: 10.1016/j.aohep.2019.04.011. Epub 2019 May 23. PMID: 31151874

6. Corso CR, **Stipp MC**, Radulski DR, Mariott M, da Silva LM, de Souza Ramos EA, Klassen G, Queiroz Telles JE, Oliveira CS, Stefanello MÉA, Verhoeven AJ, Oude Elferink RPJ, Acco A. Fruticuline A, a chemically-defined diterpene, exerts antineoplastic effects in vitro and in vivo by multiple mechanisms. Sci Rep. 2020 Oct 5;10(1):16477. doi: 10.1038/s41598-020-73432-2. PMID: 33020521; PMCID: PMC7536426.

7. Adami ER, Corso CR, Turin-Oliveira NM, Galindo CM, Milani L, **Stipp MC**, da Silva LCM, do Nascimento GE, Chaves PFP, Chequin A, Mariott M, da Silva LM, Klassen G, Ramos EAS, Cordeiro LMC, Acco A. Polysaccharides from green sweet pepper increase the antineoplastic effect of methotrexate on mammary tumor cells. Int J Biol Macromol. 2020 May 5;158:1071-1081. doi: 10.1016/j.ijbiomac.2020.05.001. Epub ahead of print. PMID: 32387356.

8. Seko, Gabriele Harumi; Mendonça, Kamila Stelly; Turin-Oliveira, Natalia Mulinari; Adami, Eliana Rezende; **Stipp, Maria Carolina**; Galindo, Claudia Martins; Corso, Claudia Rita; Milani, Letícia; Rosa, Camila Tamiello; Cordeiro, Lucimara Mach Cortês; Queiroz-Telles, José Ederaldo; Acco, Alexandra. Efeitos de polissacarídeos do jambo no modelo tumoral solido de Ehrlich em camundongos. Infarma (brasília), v. 32, p. 86, 2020.

9. **Stipp MC**, Acco A. Involvement of cytochrome P450 enzymes in inflammation and cancer: a review. Cancer Chemother Pharmacol. 2021 Mar;87(3):295-309. doi: 10.1007/s00280-020-04181-2. Epub 2020 Oct 28. PMID: 33112969