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MARIA RACHEL PEDRAZZOLI CALIXTO

CONSUMO DE CHIMARRÃO EM MULHERES DIAGNOSTICADAS COM CÂNCER DE MAMA: CORRELAÇÃO CLINICO-PATOLÓGICA E ASSOCIAÇÃO COM NÍVEIS CIRCULANTES DE CAFEÍNA E ANTIOXIDANTES

FRANCISCO BELTRÃO – PR
FEVEREIRO/2019

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Essa dissertação foi julgada adequada para obtenção do título de Mestre em Ciências Aplicadas à Saúde e aprovada em sua forma final pelo(a) Orientador(a) e pela Banca Examinadora.

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LISTA DE ABREVIATURAS E SIGLAS

ACGs – (Ácidos Clorogênicos)
AO – Ácido Oleanólico
AU – Ácido Ursólico
CA – Câncer
CAs – Cânceres
CAT - Catalase
CDI – Carcinoma Ductal Infiltrante
CEONC – Hospital de Câncer de Francisco Beltrão
CLI - Carcinoma Lobular Invasivo
CM – Câncer de Mama
EM – Erva-Mate
EROS – Espécies Reativas de Oxigênio
GSH – Glutationa Reduzida
GSH- Glutationa Reduzida
H_2O - Água
H_2O_2 - Peróxido de Hidrogênio
HER2 – Receptor 2 do fator de crescimento Epidérmico Humano
HPLC- Cromatografia Líquida De Alta Eficiência
IARC – *International Agency for Research on Cancer*
IGF-1- Fator de Crescimento Semelhante à Insulina tipo 1
IGFBP - Proteína de Ligação ao Fator de Crescimento Semelhante à Insulina
INCA – Instituto Nacional do Câncer
MCP - Proteína Quimioatrativa de Monócitos
MRM- Monitoramento De Reações Múltiplas
MT - Metalotioneína
MTs – Metalotioneínas
O_2 - Oxigênio
PMSF - Fenil-metil-sulfonil-fluoreto
RE – Receptor Estrogênio
RH- Receptores Hormonais
RP- Receptor Progesterona

SNC – Sistema Nervoso Central

TCLE- Termo De Consentimento Livre E Esclarecido

TNF - Fator de Necrose Tumoral

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Resumo

Introdução: Câncer de mama é a segunda neoplasia maligna mais comum entre as mulheres, de alta heterogeneidade clinica e biológica tornando-se uma doença complexa. Tanto fatores internos como externo ao organismo são determinantes para prevenção e progressão da doença. A *Ilex paraguariensis A*. St. Hil, um exemplar de grande interesse na Etnofarmacologia, consumido no Sul no Brasil principalmente na forma de chimarrão, possui uma composição química complexa e rica em antioxidante potencialmente explorado em diversos estudos, além de atuar na prevenção de doenças causadas por estresse oxidativo, dentre elas o câncer. Este trabalho tem como objetivo investigar o desfecho clinico-patológico do câncer de mama entre mulheres consumidoras ou não de chimarrão. **Metodologia:** Foram voluntariadas 199 mulheres atendidas no Hospital de Câncer de Francisco Beltrão (Ceonc) no período de maio de 2015 a dezembro de 2017, com lesões suspeitas para câncer de mama. Foram excluídas todas as mulheres com doença benigna de mama, totalizando um grupo de 79 mulheres portadoras de câncer de mama que apresentaram todos os dados clinico-patológicos para inclusão no estudo. O consumo de chimarrão foi caraterizado como sim ou não, o perfil antioxidante foi avaliado através da dosagem de Glutationa reduzida, metalotioneína e atividade da catalase, além de níveis de cafeína sanguíneos como parâmetros de avaliação. **Resultados e conclusão:** Em relação aos parâmetros antioxidantes das pacientes e o consumo de chimarrão houve uma diminuição da glutationa reduzida e da metalotioneína e um aumento da atividade da catalase entre as consumidoras. Os níveis de cafeína circulante das pacientes com subtipo molecular Luminal A apresentaram-se maior em comparação ao subtipo HER-2 e Luminal B, já na distribuição de acordo com o IMC observou se que aquelas com sobrepeso tinham maiores níveis de cafeína seguida das obesas comparando com as eutróficas.

Palavras-chave: (câncer de mama, antioxidantes, *Ilex paraguariensis)*

Chimarrão consumption and prognostic factors in breast cancer: Correlation with antioxidants and caffeine blood levels.

Abstract

Introduction: Breast cancer is the second most common malignant neoplasm among women, presenting high clinical and biological heterogeneity, leading to a complex disease. Both internal and external factors are determinant for the disease prevention and progression. *Ilex paraguariensis A*. St. Hil, a specimen of significant interest in ethnopharmacology, consumed in Southern Brazil mainly in the form of chimarrão, displays a complex chemical composition and is rich in antioxidants potentially exploited in several studies, in addition to acting in the prevention of diseases caused by oxidative stress, such as cancer. This study aims to investigate the clinico-pathological outcome of breast cancer women chimarrão consumers compared to non-consumers. **Methodology:** A total of 199 women treated at the Francisco Beltrão Cancer Hospital (Ceonc) were volunteers from May 2015 to December 2017 presenting suspected breast cancer lesions . All women with benign breast disease were excluded, totaling a group of 79 women presenting breast cancer who presented all the clinico-pathological data for inclusion in the study. Chimarrão consumption was categorized as positive or negative, the antioxidant profile was evaluated through the determination of reduced glutathione and metallothionein contents and catalase activity, in addition to caffeine blood level. **Results and Discussion:** Decreases in reduced Glutathione and metallothignein levels and increases in catalase activity were observed among chimarrão consumers. The levels of circulating caffeine in patients with molecular Luminal A subtype were higher in comparison to Luminal B and subtype HER-2, whereas concerning the distribution agreement with body mass index (BMI), those overweight presented higher caffeine levels than the obese women compared to eutrophic ones.

keywords: Breast cancer, antioxidants, *Ilex paraguariensis A*. St. Hil

SUMÁRIO

1. INTRODUÇÃO GERAL.....13
2. OBJETIVOS.....27
2.1 Geral..... 27
2.2 Específicos..... 27
3. METODOLOGIA 28
3.1 Desenho do Estudo 28
3.2 Coleta de dados e amostra..... 29
3.3 Extração e determinação de Glutationa Reduzida 29
3.4 Determinação da atividade da Catalase..... 29
3.5 Determinação de Metalotioneína..... 30
3.6 Dosagem de Cafeína..... 30
3.7 Análise Estatística..... 31
4. REFERÊNCIAS.....32

1. INTRODUÇÃO GERAL

Câncer é o termo genérico utilizado para caracterizar um grande grupo de doenças que podem afetar qualquer parte do organismo. A característica principal do câncer é o desenvolvimento de células anormais que crescem além de seus limites habituais, podendo invadir tecidos adjacentes do corpo e se espalhar para outros órgãos. Segundo a Organização Mundial de Saúde o câncer é uma das principais causas de morte no mundo (ORGANIZATION, 2019).

A incidência do câncer no mundo foi de 20% na última década, impactando principalmente os países de baixa e média renda (PANIS et al., 2018). Em um estudo publicado pela Agência Internacional de Pesquisa sobre Câncer (IARC), em cinco anos, a incidência de câncer em mulheres da América do Sul foi de 28,2% para câncer de mama, com mortalidade em 15,3% dos casos (CANCER, 2012)**.**

Estimativa divulgada pelo Instituto Nacional de Câncer José Alencar da Silva (INCA) para o Brasil em 2018, a ocorrência de 600 mil novos casos de câncer. Esta estimativa reflete o perfil do país para os cânceres de próstata, pulmão, mama feminina e cólon e reto como os mais incidentes, além de altas taxas de cânceres do colo do útero, estômago e esôfago (INCA, 2018). Tendo como principais fatores de risco: sedentarismo, excesso de peso, hábitos alimentares, uso de álcool e tabaco, sendo o tabagismo o fator de risco principal responsável por aproximadamente 22% dos óbitos por câncer (ORGANIZATION, 2019). Com exceção do câncer de pele não-melanoma, os tipo mais prevalentes no Brasil, são os canceres de próstata (68.220 casos novos) em homens e mama (59.700 mil) em mulheres, seguidos dos de cólon e reto, pulmão, estômago, colo do útero, cavidade oral, sistema nervoso central, leucemias e esôfago respectivamente, estimativa para 2018 (INCA, 2018). A maior incidência de câncer encontra-se nas regiões mais populosas e industrializadas do país. A Região Sul do Brasil é a de maior incidência, podendo estar relacionado aos hábitos de vida desta população e maior longevidade, além de maior número de diagnósticos e consequentemente, aumento de números na base de dados (BARROS et al., 2000; GIRIANELLI et al., 2014; PANIS et al., 2018).

O câncer de mama é a segunda neoplasia maligna mais comum entre mulheres no Brasil e no mundo, e corresponde a 28% dos casos novos de canceres a cada ano. Estima-se para o ano de 2018 no estado do Paraná a ocorrência de 64,7casos novos/100.000 habitantes (INCA, 2018). O câncer de mama é etiologia multifatorial, relacionada com a idade avançada, características reprodutivas, hereditariedade, hábitos de vida alimentares, excesso de peso e variantes ambientais (ARAÚJO DA SILVA e DA SILVA RIUL, 2011).

A idade é o principal fator de risco para o desenvolvimento do câncer de mama, acomete mulheres principalmente entre 40 a 60 anos, especialmente durante o climatério. Considerado raro antes dos 35 anos em plena atividade reprodutiva, porém está incidência vem aumentando (ARAÚJO DA SILVA e DA SILVA RIUL, 2011). Os tumores de pior prognóstico são observados em mulheres com diagnóstico abaixo de 45 anos, com uma elevada taxa de mortalidade e sobrevida menor quando comparado com pacientes no período pós-menopausa, ou com a idade acima dos 50 anos (PINHEIRO et al., 2013).

As características reprodutivas de risco para o desenvolvimento da doença são: menarca precoce (11 anos ou menos), menopausa tardia (55 anos ou mais), primeira gestação acima de 30 anos, ausência de amamentação e nuliparidade, características relacionadas com maior exposição hormonal ao estrogênio ao longo da vida (ARAÚJO DA SILVA e DA SILVA RIUL, 2011; CIBEIRA e GUARAGNA, 2006).

A obesidade, outro fator importante para o risco de desenvolvimento de câncer de mama, tem uma relação importante com o aumento do nível de estrogênio produzido no tecido adiposo, principalmente no climatério(CIBEIRA e GUARAGNA, 2006). Mecanismos que ligam o excesso de peso e câncer estão sendo compreendidos, sabe-se que a insulina e o fator de crescimento semelhante à insulina tipo 1 (IGF-1) estão envolvidos na patogênese e progressão e migração de células tumorais (BOWERS et al., 2015)**,** onde a hiperinsulinemia crônica causada pela resistência à insulina, característica da obesidade, diminui a concentração da proteína de ligação ao fator de crescimento semelhante à insulina (IGFBP), levando a um aumento da disponibilidade de IGF-1, causando alterações no ambiente celular favorecendo a formação do tumor (RENEHAN et al., 2006)**.** Além dos níveis de insulina e IGF-1, outro fator importante está relacionado com a inflamação crônica, e consequentemente o

estresse oxidativo, induzida pela obesidade, marcada pelo aumento de ácidos graxos livres circulantes e pela quimioatração de células imunes, estimulando a pela liberação de citocinas inflamatórias, incluindo interleucinas, fator de necrose tumoral (TNF) e proteína quimioatrativa de monócitos (MCP) (ELLULU et al., 2017)**.** Por fim, os efeitos do sobrepeso e obesidade associados ao risco de câncer de mama são amplamente mediados pelo aumento dos níveis de estrogênio relacionados à adiposidade, onde o tecido adiposo expressam (aumentam a atividade da enzima aromatase) enzimas metabolizadoras de hormônios esteroides, tornando-se uma importante fonte de estrogênios circulantes principalmente nas mulheres pós-menopausa, o qual aumenta a diferenciação celular e proliferação celular e inibindo a apoptose (CALLE e KAAKS, 2004).

O diagnóstico precoce é de grande importância, quando este ocorre em estágio avançado eleva as taxas de mortalidade; desta maneira, o diagnóstico precoce reduz as taxas de mortalidade, melhora a sobrevida e proporciona tratamentos mais eficazes. O diagnóstico precoce tem como objetivo identificar o câncer quando ele ainda se localiza no órgão de origem (DE CARVALHO[1] et al.).

Devido à alta heterogeneidade clínica, biológica e morfológica o câncer de mama se torna uma doença complexa (CIRQUEIRA et al., 2011; VIEIRA et al., 2008). Tumores com perfis clínicos e histológicos semelhantes apresentam prognósticos diferentes e diferentes respostas terapêuticas. (CIRQUEIRA et al., 2011; VIEIRA et al., 2008). Os adenocarcinomas correspondem a quase todas as neoplasias de mama, correspondendo à linhagem epitelial que dá origem às glândulas mamárias ou túbulos. Inicialmente, os adenocarcinomas são divididos em dois grupos: carcinoma *in situ*, que não atravessam a membrana basal durante o crescimento; e os carcinomas invasivos, que podem se metastizar para linfonodos, tecidos adjacentes ou sítios distantes. Os carcinomas também são divididos quanto à sua morfologia, em carcinoma ductal ou carcinoma lobular (MELO FILHO, 2010). Carcinomas ductal invasivo (CDI) e carcinoma lobular invasivo (CLI) são tipos histológicos mais comuns de câncer de mama. Ambos os carcinomas são derivados da unidade terminal do ducto lobular (TDLU), onde as diferenças quanto à morfologia refletem em diferenças nos mecanismos de carcinogênese, em vez da origem anatômica das lesões. Sua apresentação, os parâmetros clínicos patológicos, tais como local, tamanho, grau e estágio do

tumor são similares para ambos os tipos de tumor, no entanto, os dados de acompanhamento clínico e os padrões de metástase sugerem que seu desenvolvimento e progressão sejam diferentes (TURASHVILI et al., 2007). Em 90% dos casos os carcinomas de mama são ductais invasivos, que caracterizam uma doença bastante agressiva (MELO FILHO, 2010).

Para a classificação histológica do câncer de mama são utilizados parâmetros associados à agressividade do tumor, como grau de diferenciação, formação tubular e índice mitótico, denominados em conjunto como grau histológico. Quanto maior o grau de diferenciação tecidual, melhor o prognóstico do câncer de mama, já que tumores agressivos costumam cursar com graus histológicos indiferenciados. Outros fatores relacionados à biologia do tumor caracterizam o prognóstico e resposta terapêutica do câncer de mama, como: tamanho tumoral, presença de linfonodos positivos, presença de êmbolos angiolinfáticos e invasão de margens cirúrgicas (ARANTES JÚNIOR, 2006). No Brasil, a caracterização dos diferentes subtipos moleculares do câncer de mama é realizada através da técnica de imuno-histoquímica que avalia a expressão de receptores hormonais, receptores de estrógeno (RE) e receptores progesterona (RP), índice de proliferação celular (Ki-67) e expressão de expressão de fator epidermal humano tipo 2 (HER2) (CHEANG et al., 2009).

Desta forma, o câncer de mama se manifesta como uma doença molecularmente heterogênea que inclui pelo menos cinco subtipos moleculares principais de importância na prática clinica determinante para prognóstico e tratamento: Luminal A, Luminal B, Luminal-HER2, HER2 amplificado e Triplo-Negativo (SERRA et al., 2014). Os subtipos luminais são caracterizados pela semelhança dentre as células mamárias normais, que tem um contato direto com o lúmen dos ductos mamários, e as células deste tipo de câncer de mama, relacionando com o nome (MELO FILHO, 2010).

O subtipo Luminal A, é identificado como de melhor prognóstico (CIRQUEIRA et al., 2011; MELO FILHO, 2010; VIEIRA et al., 2008). Possui RE e/ou RP positivos, Ki-67 abaixo de 14% de células neoplásicas imunomarcadas, e a expressão de HER2 negativa(MELO FILHO, 2010).

O subtipo Luminal B, apresenta pior prognóstico que o Luminal A, menor sobrevida e maior chance de recidiva (CIRQUEIRA et al., 2011). Possui RE e/ou RP positivos, Ki-67 superior ou igual a 14% de células neoplásicas

imunomarcadas e expressão HER2 negativo (CIRQUEIRA et al., 2011; MELO FILHO, 2010).

O subtipo Luminal-HER2, também chamado de HER2-híbrido, possui RE e/ou RP positivos, e superexpressão de HER2 (SERRA et al., 2014).

O subtipo com superexpressão unicamente de HER2, também chamado de HER2 puro é classificado como o de segundo melhor prognóstico, devido à disponibilidade de terapia alvo direcionada (trastuzumabe). Apresenta negatividade tanto para RE como para RP, com superexpressão de HER2, com Ki-67 em qualquer valor (CIRQUEIRA et al., 2011).

O subtipo triplo-negativo é o subtipo de pior prognóstico, menor sobrevida, com maior agressividade e sem esquema terapêutico direcionado. Apresenta negatividade para receptores hormonais (RE e RP) e HER2 (VIEIRA et al., 2008). É caracterizado por ter um alto grau histológico, com expressão de queratinas de alto peso molecular, elevado índice de proliferação, pontos de necrose e infiltrado linfocitário (CIRQUEIRA et al., 2011). Estão inclusos nesse grupo os tumores com mutação para o gene BRCA1 e os carcinomas metaplásicos (MELO FILHO, 2010).

Em conjunto com os fatores apresentados aqui, avalia-se o prognostico da paciente com câncer de mama em relação ao seu estadiamento clínico, também denominado estadiamento TNM (do inglês, T = tamanho do tumor, N = numero de linfonodos positivos e M = presença de metástases à distância). Assim, pacientes de melhor prognostico apresentarão estadiamento inicial (I e II), e aquelas de pior prognóstico apresentarão doença com estadiamento localmente avançado ou metastático (III e IV) (GONÇALVES et al., 2012).

Apesar dos avanços no conhecimento da biologia de tumores, sabe-se que fatores externos ao organismo, como hábitos alimentares, culturais, exposição ambiental e estresse, são determinantes no curso da doença, tanto na prevenção do seu desenvolvimento como na promoção da carcinogênese (ARAÚJO DA SILVA e DA SILVA RIUL, 2011).

A *Ilex paraguariensis A*. St. Hil, também conhecida como “mate” ou “erva-mate” é uma planta da família Aquifoliaceas, nativa da América do Sul. Sua infusão é conhecida como uma bebida estimulante, com propriedades antioxidante e promotora da saúde, tradicionalmente consumida pelo Brasil, Uruguai, Argentina e Paraguai (OELLIG et al., 2018). Europa, Estados Unidos,

Síria e Japão importam folhas de erva-mate e as transformam em extratos vegetais, utilizando-as como fitoterápicos, devido sua composição rica em compostos bioativos (RIL et al., 2011). Na região sul do Brasil a erva-mate é amplamente consumida na forma de chimarrão, bebida preparada a partir de folhas verdes e secas infundida em água a quente, de sabor amargo, com propriedades estimulantes e antioxidantes (BOAVENTURA et al., 2015) em um recipiente chamado cuia (DELMONICO et al., 2015; MENDES et al., 2007).

O chimarrão foi evoluído a partir de um chá bebido pelo grupo étnico Guarani a uma bebida de caráter social com papel ritualístico nas sociedades modernas na América do Sul. A característica do consumidor do chimarrão é beber continuamente, acompanhando as atividades diárias, o consumidor chega a ingerir até 1litro a cada 1 hora. Este método de consumo permite uma extração contínua dos compostos das folhas secas. Devido a esta peculiaridade os compostos são absorvidos de forma constante durante todo o dia (BRACESCO et al., 2011) ingerida ao longo do dia o volume que pode variar de 1 a 3 litros (MENDES et al., 2007). A erva-mate possui uma composição química complexa, incluindo compostos fenólicos, alcaloides (metilxantinas) e saponinas (GERKE, 2016).

Os compostos fenólicos apresentam comportamento antioxidante, devido a sua capacidade de inibir a lipoxigenase, quelação de metais, modulação de atividade enzimática e eliminação de radicais livres. Desta maneira atuando na prevenção de diversas doenças degenerativas causadas por estresse oxidativo, dentre eles o câncer (GERKE, 2016). Os ácidos clorogênicos (CGAs), são os principais compostos fenólicos da erva-mate, entre suas propriedades eles aparentam ter uma atividade antitumoral e capacidade de inibir a carcinogênese (MIRANDA et al., 2008).

Cerca de 10% do peso total de folhas de erva-mate é constituído de saponinas triterpenoides, que confere o sabor amargo e a espuma característica do chimarrão. Os principais exemplares são o ácido ursólico (AU) e o ácido oleanólico (AO), os quais possuem propriedades quimiopreventivas (PUANGPRAPHANT et al., 2011). O ácido ursólico exerce papel em processos inflamatórios, apoptose, angiogênese e metástase, através de múltiplos alvos intracelulares e extracelulares. Tanto o ácido oleanólico como o ácido ursólico

desempenham uma atividade inibidora de aromatase, essa característica pode explicar em parte sua propriedade antitumoral (RONCO et al., 2017).

Os alcaloides vegetais cafeína, teofilina e teobromina são importantes componentes ativos, derivados da xantina e também conhecidos como metilxantinas (OELLIG et al., 2018). A cafeína (1,3,7- trimetilxantina), considerada um dos principais componentes da erva-mate, é o mais comum dentre os três, sendo encontrada principalmente em chás, cafés, produtos de cacau e bebidas à base de cola (ALVES e BRAGAGNOLO, 2002). É o psicoestimulante mais utilizado nos países ocidentais, com propriedades antioxidantes, anti-inflamatórias e antiapoptótica (KOLAHDOUZAN e HAMADEH, 2017). Possui uma acentuada característica psicoestimulante após a ingestão de doses baixas e moderadas (50-300 mg), promovendo o estado de alerta, a capacidade de concentração e melhorando a atividade cognitiva (ALVES et al., 2009). Alguns efeitos fisiológicos do consumo de cafeína incluem estimulação do sistema nervoso central, sistema cardiovascular e respiratório, além da secreção de ácido gástrico, relaxamento muscular e atividade diurética (ALVES e BRAGAGNOLO, 2002). Assim, a concentração de cafeína, teofilina e teobromina na erva mate geralmente é de grande interesse para o entendimento dos efeitos do consumo desta planta (OELLIG et al., 2018).

Em relação aos antioxidantes presentes na erva-mate, além de saponinas, cafeína e teobromina, a infusão de erva mate é rica em vitamina A, vitamina E e vitamina C, tiamina, riboflavina, niacina, potássio, magnésio, cálcio, manganês, ferro selênio, fosforo, zinco e polifenóis. Ainda são encontrados compostos voláteis derivados do ácido caféico, como o ácido clorogênico e flavonoides (FAGUNDES et al., 2015).

Mecanismos de ação estão presentes em produtos que em sua composição apresentam ácidos fenólicos e flavonoides atuando no organismo como agente antioxidante, tendo a capacidade de agir como protetores frente ao processo de oxidação (BRACESCO et al., 2011). A erva mate apresenta uma capacidade de varrer espécies reativas de oxigênio (EROs) semelhante á da enzima antioxidantes peroxidase. Essa atividade é dose dependente relacionada com a concentração de polifenóis da erva mate (ANESINI et al., 2006). Estudos comprovam os benefícios à saúde proporcionados pelo consumo de bebidas à

base de erva-mate, entre eles melhora a defesa do organismo contra radicais livres (DOS SANTOS et al., 2014; SCHINELLA et al., 2000).

Além da sua capacidade antioxidante, outras atividades biológicas merecem destaque, em especial seus efeitos anticarcinogênicos. Em testes *in vitro*, mostrou ser capaz de inibir a topoisomerase II, que é responsável pela divisão celular e pela inibição da proliferação de células cancerígenas, e também ser um potente inibidor de proteossoma, o qual tem sido investigado como um possível meio de tratamento do câncer (HECK e DE MEJIA, 2007).

Atualmente, está bem estabelecido que o estresse oxidativo esteja envolvido em vários estados patológicos, como câncer, distúrbios cardiovasculares, diabetes, artrite, inflamação e doenças hepáticas (MIRANDA et al., 2008)**.**

O estresse oxidativo é o resultado patogênico resultante do desequilíbrio entre a produção de Espécies reativas de oxigênio e a capacidade antioxidante das células (LANDRISCINA et al., 2009). As espécies reativas de oxigênio (EROS) são fisiologicamente produzidas pelo organismo por células aeróbicas. Sob condições de lesão celular sua produção é aumentada, gerando dano celular e morte (TRAVERSO et al., 2013). Portanto para prevenir o dano celular, o aumento de EROS induz uma resposta adaptativa compensatória dos sistemas antioxidantes, que visa restaurar a homeostase redox, equilíbrio entre a taxa de geração de oxidantes e a taxa de desintoxicação oxidante, (LANDRISCINA et al., 2009)**.**

A Glutationa reduzida (GSH) desempenha uma importante função de manutenção intracelular do equilíbrio redox, tornando-se um dos principais e mais abundantes antioxidantes endógenos em quase todas as células humanas (SEN e PACKER, 1996)**.** O aumento dos níveis de GHS na célula está associado à resposta proliferativa sendo essencial para a progressão do ciclo celular tanto em células normais como tumorais (COSTANTINO et al., 2009)**.** Sendo assim, foi demonstrado que níveis de elevados de GSH estão relacionados de forma direta tanto com a proliferação celular como também em atividade metastática de células tumorais (LANDRISCINA et al., 2009).

As Metalotioneínas (MTs) pertecem ao grupo das proteínas não enzimática e citosólicas cuja estão envolvidas em diversas funções intracelulares, sendo a

homeostase de metais essenciais, como o Zinco e Cobre que são essenciais para a proliferação e diferenciação celular, e a desintoxicação de metais pesados devido à alta afinidade por estes metais (RUTTKAY-NEDECKY et al., 2013) O papel das MTs está relacionado com a proteção contra o estresse oxidativo, sendo de grande importância na remoção de EROS (WERNER et al., 2008)**,** atua contra radicais livres gerados por citotoxicidade, agentes mutagênicos, antineoplásicos, e radiação, além de protetor contra danos de DNA e apoptose (SI e LANG, 2018)**.** As MTs geralmente são expressas em baixos níveis, porem induzíveis. Sua síntese se demonstra aumentada durante o estresse oxidativo (GUMULEC et al., 2014).

A Catalase (CAT) é uma enzima antioxidante com a clássica atividade de metabolizar peróxido de hidrogênio (H_2O_2) em água (H_2O) e oxigênio (O_2), paralelamente a sua atividade clássica, também decompõe o peroxinitrito e pode oxidar o óxido nítrico em dióxido de nitrogênio (GLORIEUX et al., 2014)**.** É altamente expressa em alguns tecidos humanos, e desempenha o papel de proteger as células contra os danos causados pelo excesso e formação de EROS (GLORIEUX et al., 2011)**.** Sendo o peróxido de hidrogênio e o ânion superóxido como principais fontes de EROs endógenos gerados como subproduto do metabolismo celula (WARIS e AHSAN, 2006).

As células eucarióticas são constantemente atacadas por espécies reativas de oxigênio (EROs), subprodutos naturais do metabolismo energético celular que podem ser formados em grandes quantidades por exercício exaustivo ou por agentes químicos presentes no ambiente e em alimentos. O dano oxidativo ao DNA por EROs resulta em modificações das bases do DNA, quebras simples e duplas e formação de lesões apurínicas/apirimidínicas pró-mutagênicas, que se não forem reparados, podem resultar em mutações (MIRANDA et al., 2008). Desta forma, defesas endógenas antioxidantes nem sempre conseguem neutralizar completamente as EROS, desta maneira derivados da dieta, parecem ser de grande importância na proteção, melhorando o estado antioxidante principalmente nas doenças crônicas (YANG e LIU, 2013)**.** Conforme citado, na *Ilex paraguariensis*, há vários compostos bioativos rico em antioxidantes com

propriedades moduladoras redox, associados na grande maioria às defesas contra EROS (COLPO et al., 2017).

Poucos estudos investigaram a relação do consumo de erva-mate e câncer de mama. No estudo caso-controle de RONCO et al. (2017), em que foram avaliados 572 casos de câncer de mama e 889 controles em relação ao consumo de erva mate, os autores encontraram associação inversa entre alta ingestão de erva mate e o risco de desenvolvimento de câncer de mama com relação aos fatores de risco reprodutivos (menarca precoce, nuliparidade, pouca amamentação, e elevados números de ciclos ovulatórios). Esta associação foi mais forte em mulheres pós-menopausadas que possuíam uma dieta de alto conteúdo calórico (≤1945 kcal/ dia).

Em outro estudo publicado por RONCO, A. L. et al. (2016) com objetivo de avaliar a associação da ingestão de erva-mate com risco de câncer de mama, foram analisados parâmetros relacionados ao consumo da erva-mate (quantidade diária ingerida, duração do hábito e intensidade do consumo). Foram encontrados os seguintes resultados: consumidores atuais mostraram uma redução significativa de risco para câncer de mama, dose diária superior a 1 litro de infusão foi fortemente protetora, a duração do hábito sugeriu o benefício do consumo em longo prazo, e a intensidade de consumo também mostrou forte associação inversa em comparação com o grupo de não consumidores. Além de outras analises que demonstraram que o consumo de mate teve um afeito protetor em mulheres que tinham um alto consumo calórico, alto índice de massa corporal e nas menopausadas sugerindo evidências de uma associação inversa significativa para o consumo de mate e risco de câncer de mama.

Com objetivo de estudar uma associação positiva entre o consumo de erva-mate e câncer, STEFANI et al. (2011) analisou durante o período de 1990-2004, 8.875 casos de câncer confirmados de boca, faringe, esôfago, estômago, cólon, reto, laringe, pulmão, mama feminina, colo uterino, próstata, bexiga, e rim. Os maiores riscos para consumidores de erva-mate foram observados para canceres de esôfago, pulmão e bexiga, enquanto câncer de boca, faringe, cólon, reto e mama feminina não foram associados ao consumo. Em relação ao consumo em longo prazo de erva mate, câncer do trato aerodigestivo superior (cavidade bucal, hipofaringe e parte inferior da faringe), esôfago, laringe, pulmão,

próstata, bexiga e rim foram positivamente associados ao consumo de mate por mais de 50 anos. Estes resultados sugerem que produtos químicos, como o benzo (a) pireno, podem ser responsáveis pelo efeito carcinogênico do mate nos locais de câncer mencionados acima. Quanto aos efeitos da temperatura do consumo da erva mate, câncer de estomago e cólon não foram associados com o consumo do mate quente, enquanto câncer de pulmão, esôfago e bexiga foram fortemente associados ao consumo do mate quente.

Outro estudo explorou a possível associação entre a ingestão de mate e chá, antioxidantes e risco de câncer de mama, onde a maior ingestão de mate foi significante e inversamente associada ao risco de câncer de mama independente dos níveis de antioxidantes da dieta, entre eles os carotenoides, vitamina C, vitamina E, flavonoides e glutationa. Encontrando evidências do efeito protetor do mate sobre o risco de câncer de mama (RONCO, ALVARO L et al., 2016).

A cafeína é de longe o composto da erva-mate com mais estudos publicados. Em um estudo prospectivo de coorte com 85.987 mulheres, no período de 1980 a 2002, em que 5.272 eram portadoras de câncer de mama, foi encontrada uma fraca associação inversa, porém significante entre o consumo médio de café e o risco de desenvolvimento de cânceres de mama RE e RP positivos. Também se observou que a cafeína está associada a um risco menor de câncer de mama na pós-menopausa do que nos cânceres de mama na pré-menopausa; e esta associação foi mais forte com câncer de mama positivo para RE e RP do que câncer de mama negativo para RE e RP, sugerindo um possível papel envolvendo hormônios esteroides. Estes resultados sugerem que a cafeína pode estar inversamente associada ao risco de desenvolvimento de câncer de mama na pós-menopausa, particularmente em um ambiente de baixo estrogênio, propiciando o desenvolvimento de tumores de melhor prognostico (GANMAA et al., 2008).

Em um estudo sueco, foi avaliado o consumo de café e chá, e consequentemente a ingestão de cafeína, em 1.395 mulheres com casos confirmados de câncer de mama. Onde neste estudo prospectivo encontramos uma tendência decrescente significativa de câncer de mama com o aumento do consumo de café e consequentemente de cafeína. Analisando o consumo de chá, e consequente diminuição do consumo de cafeína, houve uma associação positiva entre o consumo de chá e o câncer de mama. Em conclusão estes

achados sugerem que o consumo de café e cafeína não está associado com o risco de câncer de mama ER e EP positivos, enquanto o consumo de chá pode ser positivamente associado (OH et al., 2015).

ROSENDAHL et al. (2015) com o objetivo de investigar como o café pode afetar o crescimento do câncer de mama em relação ao status do RE, identificou curiosamente que o moderado e elevado consumo de café foi associado com tumores significativamente menores, com menor proporção de RE positivos e uma tendência não significativa para menor proliferação tumoral medida pelo índice Ki-67 comparados com pacientes que consumiam pouco café. Estes resultados sugerem que o café pode ter propriedades de supressão do crescimento em células de câncer de mama e que os tumores ER positivos podem ser mais sensíveis aos efeitos causados pelo café. A nível celular o aumento das doses de cafeína suprimiu significativamente a proliferação e número total de células de câncer de mama RE positivo, o ácido cafeico também reduziu o crescimento celular, embora em menor grau que a cafeína. Em relação aos RE, a cafeína reduziu significativamente a abundância de RE nas células RE positiva. Quanto ao prognóstico do câncer de mama, a sobrevida livre de doença foi significativamente melhorada entre mulheres tratadas com tamoxifeno com tumores RE positivo que bebem 2 ou mais xícaras de café ao longo do dia, entre estas mulheres houve um risco diminuído de 49% de recorrência precoce do câncer de mama ao consumo moderado a alto de café em comparação com o baixo consumo de café (ROSENDAHL et al., 2015).

Em uma meta-analise publicada em 2013 resumiu as evidências de estudos sobre a associação de café e cafeína com o risco de câncer de mama, onde 37 artigos foram selecionados, incluindo 59.018 casos de câncer de mama. No geral, 20 estudos incluindo 41.805 casos de câncer de mama, verificaram que o consumo de café diminui o risco de câncer de mama em 2% a cada 2 xícaras de café consumidas no dia. Para estudos caso controle, 8 estudos foram selecionados onde uma relação linear foi encontrada entre a ingestão de café e o risco de câncer de mama, diminuindo o risco de câncer de mama entre 3% a cada 2 xícaras de café consumidas ao dia. Para estudos de coorte, dados de 12 estudos foram incluídos, uma relação linear foi encontrada entre a ingestão do café e risco de câncer de mama, onde o risco de câncer de mama, também, diminui em 2% cada 2 xícaras de café consumidas no dia. Para a associação de

cafeína com câncer de mama, 7 estudos foram incluídos com 14.020 casos de câncer de mama, onde foram encontrados uma relação linear entre o consumo de cafeína e risco de câncer de mama, diminuindo o risco de câncer de mama em 1% a cada 200mg de ingestão de cafeína por dia (JIANG et al., 2013).

Em outro estudo prospectivo no qual 110.454 mulheres foram incluídas, foram avaliados fatores de risco para desenvolvimento de câncer de ovário, observou-se uma associação inversa entre a ingestão de cafeína e risco para esta neoplasia, principalmente em mulheres que não usavam hormônios. Estes achados sugerem que a cafeína consumida ao longo da vida pode influenciar o desenvolvimento do câncer de modo a favorecer o desenvolvimento de neoplasias menos agressivas (TWOROGER et al., 2008).

No entanto, apesar de inúmeros estudos apontarem para um efeito protetor, existem trabalhos indicando resultados conflitantes neste contexto. Um estudo com uma coorte de 39.532 mulheres com tempo médio de seguimento aproximadamente 12,2 anos, verificou que o café, chá e cafeína não foram associados com o risco global de câncer de mama e de ovário. Houve, no entanto, uma tendência para um aumento do risco de câncer de mama com o aumento do consumo de café com ou sem cafeína e/ou cafeína entre mulheres na pré-menopausa e com peso normal. Os autores sugerem mais estudos para confirmar estes resultados (ARTHUR et al., 2018).

No estudo caso-controle, conduzido por BISSONAUTH et al. (2009) no Canadá, no qual foram avaliados o consumo de calorias, café e álcool em uma corte de 560 pacientes com câncer de mama. Observaram que as mulheres que bebiam mais de oito xícaras de café por dia tiveram um risco aumentado de câncer de mama, evidenciando que o consumo do café pode desempenhar um papel no risco no desenvolvimento desse câncer.

Uma associação entre o consumo de metilxantinas e o risco de câncer de mama foi avaliado em um estudo caso-controle no qual incluiu 451 casos de câncer de mama. No geral, dentro dos casos pós-menopausa, houve pouca variação no risco de câncer de mama em relação a cafeína consumida. Portanto nas mulheres pré-menopausa houve uma pequena variação onde o risco de câncer de mama foi associado com a ingestão de cafeína, o risco aumentou linearmente conforme o aumento da ingestão (ROHAN e MCMICHAEL, 1988).

Apesar de a erva-mate apresentar-se como uma excelente fonte de compostos antioxidantes e antitumorais, a literatura carece de estudos que comprovem o efeito do seu consumo ao longo da vida em pacientes com câncer de mama. Assim, neste estudo investigou-se o perfil clinico-patológico do câncer de mama desenvolvido por mulheres residentes no Sudoeste do Paraná atendido pelo Hospital de Câncer de Francisco Beltrão, consumidoras ou não de chimarrão. Além de avaliar a existência de associação entre os desfechos clínicos e os níveis de cafeína e antioxidantes no sangue periférico.

2. OBJETIVOS

2.1 Geral

Investigar o desfecho clinico-patológico do câncer de mama entre mulheres consumidoras ou não de chimarrão ao longo da vida, e avaliar se existe correlação com os níveis de cafeína e antioxidantes

2.2 Específicos

- Investigar o padrão de consumo de chimarrão em uma população de mulheres diagnosticada com câncer de mama atendidas no Hospital de Câncer de Francisco Beltrão;
- Mapear o perfil clinico-patológico da população estudada, através da coleta de dados de prontuários médicos;
- Realizar dosagem dos níveis de cafeína em amostras de plasma destas pacientes;
- Investigar se o consumo de chimarrão é capaz de alterar a capacidade antioxidante sanguínea destas pacientes;
- Estabelecer se existe correlação clinico-patológica entre o consumo de chimarrão, níveis de cafeína, antioxidantes sanguíneos e parâmetros clinico-patológicos que determinam o prognóstico do câncer de mama.

3. METODOLOGIA

3.1 Desenho do Estudo

Neste estudo foram voluntariadas 199 mulheres atendidas no Hospital de Câncer de Francisco Beltrão (Ceonc) no período de maio de 2015 a dezembro de 2017, com lesões suspeitas para câncer de mama. Após confirmação do diagnóstico, as mesmas foram categorizadas como portadoras de doenças benignas da mama ou carcinoma ductal infiltrante de mama. Foram excluídas do estudo todas as mulheres com doença benigna de mama, totalizando um grupo de 79 mulheres portadoras de câncer de mama que apresentaram todos os dados clinico-patológicos para inclusão no estudo. Todas as mulheres assinaram termos de consentimento livre e esclarecido (TCLE). Esta pesquisa foi aprovada pelo Comitê de Ética em Pesquisa com Seres Humanos (CAAE 35524814.4.0000.0107).

O consumo de chimarrão foi categorizado como sim ou não, e devido à dificuldade de se estabelecer o padrão de consumo em litros por dia, decidiu-se avaliar o perfil antioxidante e níveis de cafeína sanguíneos como parâmetros de avaliação.

Para caracterização clinico-patológica dos grupos foram considerados os seguintes parâmetros: idade no diagnóstico da doença, peso, altura, classificação internacional de tumores de mama TNM para estadiamento, status hormonal dos tumores, tipo de regime quimioterápico instituído, categorização de risco das pacientes, status linfonodal e presença ou não de menopausa. Para a subtipagem dos tumores, foi realizada marcação imuno-histoquímica para os receptores de estrógeno (ER), progesterona (PR), índice de proliferação ki67 e do receptor do fator de crescimento epidermal humano 2 (HER2). Com base nesta imunomarcação, os tumores foram categorizados em tumores Luminal A (ER e/ou PR positivos), Luminal B (ER e/ou PR positivos, e/ou HER2 positivos, e/ou ki 67 >14%), Luminal HER (ER e/ou PR positivos, HER2 positivos), HER2 (ER e PR negativos, HER2 positivos) e triplo negativos (todas marcações negativas, independentemente dos valores de ki67).

3.2 Coleta de dados e amostra

Foi realizada coleta de amostras de sangue periférico heparinizado (10 mL) para obtenção de plasma após centrifugação (4000 rpm, 5 minutos). O plasma foi armazenado a -20 ^{0} C até o momento das análises.

3.3 Extração e determinação de Glutationa Reduzida

A extração de glutationa reduzida seguiu o protocolo de BEUTLER (1975), com modificações por WILHELM FILHO et al. (2005). Alíquotas de 150 uL de cada amostra foram pipetadas em microtubos de polipropileno estéreis com capacidade para 2 mL e homogeneizadas em uma solução tampão fosfato de sódio 0,1 mol L^{-1} em pH 6,5, contendo sacarose 0,25 mol L^{-1} e EDTA 1 mmol L^{-1}. As amostras foram então centrifugadas em centrífuga refrigerada (5430 R, Eppendorf) à 11.000 *x g* a 4 ºC por 30 minutos. Os sobrenadante finais foram então transferidos para novos microtubos e congelados em ultra-freezer a -80ºC até o momento da análise. A determinação de GSH nos sobrenadantes foi realizada pelo método espectrofotométrico através da reação ELLMAN (1959) a 412 nm, usando uma curva analítica plotada com GSH como padrão externo.

3.4 Determinação da atividade da Catalase

A atividade de catalase foi ensaiada de acordo com o método descrito por LUSHCHAK et al. (2005). O meio reacional foi composto por 1,0 mM de EDTA, 20 mM de H_2O_2 em solução tampão fosfato de sódio 0,1 M, pH 7,5. O consumo de H_2O_2 foi continuamente registrado no espectrofotômetro (λ = 240 nm) a 25 ºC. Os cálculos para determinar a atividade enzimática foram feitos usando o coeficiente de absortividade milimolar do H_2O_2 (ε_{240} = 43,6 $mM^{-1}cm^{-1}$).

3.5 Determinação de Metalotioneína

As Metalotioneínas foram extraídas por extração térmica seguindo o protocolo de ERK et al. (2002) com adaptações. Este processo consistiu na pesagem de 150 uL de cada amostra em micro-tubos de polipropileno estéreis com capacidade para 2 mL, seguidos de homogeneização em solução tampão TRIS-HCl em pH 8,6, contendo fenil-metil-sulfonil-fluoreto (PMSF) como inibidor de protease e β-mercapoetanol como agente redutor em vórtex, por 1 minuto. Subsequentemente, as amostras centrifugadas à 20.000 x g a 4 ºC durante 60 minutos em uma centrífuga refrigerada (5430 R, Eppendorf). Após a centrifugação, os sobrenadantes foram transferidos para outros micro-tubo com capacidade para 2 mL e aquecidos em uma chapa de aquecimento a 70 ºC durante 10 minutos. Após o aquecimento, as amostras foram centrifugadas novamente à 20.000 x g a 4 ºC por 30 minutos. Os sobrenadantes finais purificados foram então vez transferido para novos micro-tubos e congeladas em ultra-freezer a -80 ºC até o momento da análise. A determinação de MT nos sobrenadantes foi realizada pelo método espectrofotométrico através da reação de Ellman a 412 nm (Ellman, 1959), usando uma curva analítica plotada com GSH como padrão externo, estimando os níveis de MT pela relação 1 mol MT = 20 mol GSH (KAGI, 1991).

3.6 Dosagem de Cafeína

A cafeína foi dosada a partir da técnica HPLC (Cromatografia líquida de alta eficiência) no plasma das amostras coletadas. O sistema consistiu em um aparelho Shimadzu HPLC (Shimadzu Scientific Instruments, Norcross, GA, EUA), acoplado com um amostrador automático HTC PAL (Leap Technologies, CTC Analytics, Carrboro, NC). A separação cromatográfica de cafeína, e do padrão interno foi realizada em coluna C18 (150 milímetros x 3mm, Phenomenex, Torrance, CA) com um gradiente de eluição, utilizando misturas de água e metanol, fase móvel A (95: 5 , v / v) e a fase móvel B (10:90, v / v), ambas contendo ácido fórmico a 0,05%, descrito por SCHREIBER-DETURMENY e

BRUGUEROLLE (1996). A detecção foi realizada utilizando um espectrômetro de massa triplo quadrupolo modelo API MDS Sciex 3000 (Applied Biosystems, Foster City, CA). Todas as análises foram realizadas no modo de monitoramento de reações múltiplas (MRM). Instrumento de controle e aquisição de dados será realizada utilizando o pacote de software Analyst (Applied Biosystems, Foster City, CA). A preparação na amostra da cafeína da amostra foi realizada por precipitação das proteínas com metanol. Alíquotas de 50 microlitros de plasma, amostras de calibração, amostras de controle de qualidade foram transferidos para tubos 0,5 mL de microcentrífuga; 175 μL de metanol gelado, contendo 10 ml do interno padrão (10 ng/mL) será adicionado a cada tubo. As amostras foram brevemente misturadas no vórtex a velocidade elevada e mantidos sobre gelo durante 40 minutos. As amostras foram então centrifugadas a 14000 G, durante 10 min a 4 ° C. Cerca de 120 μL do sobrenadante de cada tubo foram transferidos para um frasco amostrador automático, âmbar, limpo com insert para análise. Uma alíquota de 10 μLl da solução foi injetada no sistema LC-MS / MS. O ensaio desenvolvido foi validado para linearidade, exatidão, precisão e recuperação.

3.7 Análise Estatística

As análises foram realizadas em duplicata, e os dados expressos em mediana (para dados não paramétricos) ou médias (para dados paramétricos) ± erro padrão da média. Os resultados foram analisados usando o teste de Grubbs para detecção de outliers, e nenhum outlier foi detectado neste estudo. Os resultados foram comparados pelo teste t de Student ou pelo teste de Mann-Whitney, de acordo com a distribuição das variâncias, considerando $p < 0,05$. Todas as análises estatísticas foram realizadas usando o GraphPad Prism versão 5.0 (GraphPad Software, San Diego, CA, EUA). Para obtenção das frequências, teste de Qui-quadrado e regressão logística, utilizou-se o software SPSS 22.0.

4. REFERÊNCIAS

ALVES, A. B.; BRAGAGNOLO, N. Determinação simultânea de teobromina, teofilina e cafeína em chás por cromatografia líquida de alta eficiência. **Revista Brasileira de Ciências Farmacêuticas,** v. 38, n. 2, p. 237-243, 2002.

ALVES, R. C.; CASAL, S.; OLIVEIRA, B. Benefícios do café na saúde: mito ou realidade. **Quim. Nova,** v. 32, n. 8, p. 2169-2180, 2009.

ANESINI, C.; FERRARO, G.; FILIP, R. Peroxidase-like activity of Ilex paraguariensis. **Food Chemistry,** v. 97, n. 3, p. 459-464, 2006.

ARANTES JÚNIOR, J. C. Perfis Histopatológico e Imuno-histoquímico do câncer de mama: Comparação entre lesões palpáveis e não-palpáveis. 2006.

ARAÚJO DA SILVA, P.; DA SILVA RIUL, S. Câncer de mama: fatores de risco e detecção precoce. **Revista Brasileira de Enfermagem,** v. 64, n. 6, 2011.

ARTHUR, R.; KIRSH, V. A.; ROHAN, T. E. Associations of coffee, tea and caffeine intake with risk of breast, endometrial and ovarian cancer among Canadian women. **Cancer Epidemiol,** v. 56, p. 75-82, Oct 2018.

BARROS, S. G. S. D. et al. Mate (chimarrão) é consumido em alta temperatura por população sob risco para o carcinoma epidermóide de esôfago. **Arquivos de gastroenterologiay. São Paulo. Vol. 37, n. 1 (jan./mar. 2000), p. 25-30**, 2000.

BEUTLER, E. **Red cell metabolism: a manual of biochemical methods**. Grune & Stratton, 1975. ISBN

BISSONAUTH, V. et al. Risk of Breast Cancer Among French-Canadian Women, Noncarriers of More Frequent BRCA1/2 Mutations and Consumption of Total Energy, Coffee, and Alcohol. **The Breast Journal,** v. 15, n. s1, p. S63-S71, 2009.

BOAVENTURA, B. C. B. et al. Effect of yerba mate (Ilex paraguariensis A. St. Hil.) infusion obtained by freeze concentration technology on antioxidant status of healthy individuals. **LWT-Food Science and Technology,** v. 62, n. 2, p. 948-954, 2015.

BOWERS, L. W. et al. The Role of the Insulin/IGF System in Cancer: Lessons Learned from Clinical Trials and the Energy Balance-Cancer Link. **Front Endocrinol (Lausanne),** v. 6, p. 77, 2015.

BRACESCO, N. et al. Recent advances on Ilex paraguariensis research: minireview. **J Ethnopharmacol,** v. 136, n. 3, p. 378-84, Jul 14 2011.

CALLE, E. E.; KAAKS, R. Overweight, obesity and cancer: epidemiological evidence and proposed mechanisms. **Nat Rev Cancer,** v. 4, n. 8, p. 579-91, Aug 2004.

CANCER, I. A. F. R. O. Estimated cancer incidence, mortality and prevalence worldwide in 2012. 2012. Acesso em: June.

CHEANG, M. C. et al. Ki67 index, HER2 status, and prognosis of patients with luminal B breast cancer. **JNCI: Journal of the National Cancer Institute,** v. 101, n. 10, p. 736-750, 2009.

CIBEIRA, G. H.; GUARAGNA, R. M. Lipídio&58; fator de risco e prevenção do câncer de mama Lipid&58; risk factor and breast cancer prevention. **Revista de nutrição,** v. 19, n. 1, p. 65-75, 2006.

CIRQUEIRA, M. B. et al. Subtipos moleculares do câncer de mama. **Femina,** v. 39, n. 10, 2011.

COLPO, A. C. et al. Compounds from Ilex paraguariensis extracts have antioxidant effects in the brains of rats subjected to chronic immobilization stress. **Applied Physiology, Nutrition, and Metabolism,** v. 42, n. 11, p. 1172-1178, 2017.

COSTANTINO, E. et al. TRAP1, a novel mitochondrial chaperone responsible for multi-drug resistance and protection from apoptotis in human colorectal carcinoma cells. **Cancer Lett,** v. 279, n. 1, p. 39-46, Jun 28 2009.

DE CARVALHO[1], P. L. et al. Qualidade de vida de mulheres em tratamento de câncer de mama.

DELMONICO, L.; ALVES, G.; DO AMARAL, L. F. A biologia do câncer de mama e testes moleculares de prognóstico. **Revista Hospital Universitário Pedro Ernesto,** v. 14, 2015.

DOS SANTOS, C. O. et al. Caracterização, teor de polifenóis totais e atividade antioxidante em diferentes tipos de erva-mate (Ilex paraguariensis St. Hill.) para chimarrão. **Revista do Instituto Adolfo Lutz,** v. 73, n. 1, p. 77-86, 2014.

ELLMAN, G. L. Tissue sulfhydryl groups. **Arch Biochem Biophys,** v. 82, n. 1, p. 70-7, May 1959.

ELLULU, M. S. et al. Obesity and inflammation: the linking mechanism and the complications. **Arch Med Sci,** v. 13, n. 4, p. 851-863, Jun 2017.

ERK, M. et al. Evaluation of different purification procedures for the electrochemical quantification of mussel metallothioneins. **Talanta,** v. 57, n. 6, p. 1211-1218, 2002.

FAGUNDES, A. et al. Ilex Paraguariensis: composto bioativos e propriedades nutricionais na saúde. **RBONE-Revista Brasileira de Obesidade, Nutrição e Emagrecimento,** v. 9, n. 53, p. 213-222, 2015.

GANMAA, D. et al. Coffee, tea, caffeine and risk of breast cancer: a 22-year follow-up. **Int J Cancer,** v. 122, n. 9, p. 2071-6, May 1 2008.

GERKE, I. B. B. Extrato aquoso de erva-mate (Ilex paraguariensis St. Hill) clarificado: extração em batelada e processamento por membranas. 2016.

GIRIANELLI, V.; GAMARRA, C.; AZEVEDO E SILVA, G. Os grandes contrastes na mortalidade por câncer do colo uterino e de mama no Brasil. **Rev Saude Publica,** v. 48, n. 3, p. 459-67, 2014.

GLORIEUX, C. et al. Catalase expression in MCF-7 breast cancer cells is mainly controlled by PI3K/Akt/mTor signaling pathway. **Biochem Pharmacol,** v. 89, n. 2, p. 217-23, May 15 2014.

GLORIEUX, C. et al. Catalase overexpression in mammary cancer cells leads to a less aggressive phenotype and an altered response to chemotherapy. **Biochem Pharmacol,** v. 82, n. 10, p. 1384-90, Nov 15 2011.

GONÇALVES, L. L. C. et al. Câncer de mama feminino: aspectos clínicos e patológicos dos casos cadastrados de 2005 a 2008 num serviço público de oncologia de Sergipe. **Revista Brasileira de Saúde Materno Infantil,** v. 12, n. 1, p. 47-54, 2012.

GUMULEC, J. et al. Metallothionein - immunohistochemical cancer biomarker: a meta-analysis. **PLoS One,** v. 9, n. 1, p. e85346, 2014.

HECK, C. I.; DE MEJIA, E. G. Yerba Mate Tea (Ilex paraguariensis): a comprehensive review on chemistry, health implications, and technological considerations. **J Food Sci,** v. 72, n. 9, p. R138-51, Nov 2007.

INCA. **Estimativa 2018. Incidência de câncer no Brasil [Internet]. Instituto Nacional de Câncer José Alencar Gomes da Silva. 2017. 130 p** 2018.

JIANG, W.; WU, Y.; JIANG, X. Coffee and caffeine intake and breast cancer risk: an updated dose-response meta-analysis of 37 published studies. **Gynecol Oncol,** v. 129, n. 3, p. 620-9, Jun 2013.

KAGI, J. H. Overview of metallothionein. **Methods Enzymol,** v. 205, p. 613-26, 1991.

KOLAHDOUZAN, M.; HAMADEH, M. J. The neuroprotective effects of caffeine in neurodegenerative diseases. **CNS neuroscience & therapeutics,** v. 23, n. 4, p. 272-290, 2017.

LANDRISCINA, M. et al. Adaptation to oxidative stress, chemoresistance, and cell survival. **Antioxid Redox Signal,** v. 11, n. 11, p. 2701-16, Nov 2009.

LUSHCHAK, V. I. et al. Hypoxia and recovery perturb free radical processes and antioxidant potential in common carp (Cyprinus carpio) tissues. **Int J Biochem Cell Biol,** v. 37, n. 6, p. 1319-30, Jun 2005.

MELO FILHO, A. F. AVALIAÇÃO POR IMUNOISTOQUÍMICA DA EXPRESSÃO DE FATORES DE CRESCIMENTO E DE FATORES RELACIONADOS À INFLAMAÇÃO EM CÂNCER DE MAMA TRIPLO-NEGATIVO. 2010.

MENDES, R. M. D. O.; QUADRI, M. B.; QUADRY, M. G. N. Extraneous material in mate (Ilex paraguariensis St Hil) industrialized in Santa Catarina state, Brasil. **Revista do Instituto Adolfo Lutz (Impresso),** v. 66, n. 2, p. 103-107, 2007.

MIRANDA, D. D. et al. Protective effects of mate tea (Ilex paraguariensis) on H2O2-induced DNA damage and DNA repair in mice. **Mutagenesis,** v. 23, n. 4, p. 261-5, Jul 2008.

OELLIG, C.; SCHUNCK, J.; SCHWACK, W. Determination of caffeine, theobromine and theophylline in Mate beer and Mate soft drinks by high-performance thin-layer chromatography. **Journal of Chromatography A,** v. 1533, p. 208-212, 2018.

OH, J. K. et al. Prospective study of breast cancer in relation to coffee, tea and caffeine in Sweden. **International journal of cancer,** v. 137, n. 8, p. 1979-1989, 2015.

ORGANIZATION, W. H. Cancer. p. https://www.who.int/cancer/en/, 2019. Acesso em: June.

PANIS, C. et al. Critical review of cancer mortality using hospital records and potential years of life lost. **Einstein (São Paulo),** v. 16, n. 1, 2018.

PINHEIRO, A. B. et al. Câncer de mama em mulheres jovens: análise de 12.689 casos. **Rev. Bras. Cancerol.(Online),** v. 59, n. 3, p. 351-359, 2013.

PUANGPRAPHANT, S.; BERHOW, M. A.; DE MEJIA, E. G. Mate (Ilex paraguariensis St. Hilaire) saponins induce caspase-3-dependent apoptosis in human colon cancer cells in vitro. **Food Chemistry,** v. 125, n. 4, p. 1171-1178, 2011.

RENEHAN, A. G.; FRYSTYK, J.; FLYVBJERG, A. Obesity and cancer risk: the role of the insulin-IGF axis. **Trends Endocrinol Metab,** v. 17, n. 8, p. 328-36, Oct 2006.

RIL, F. T. et al. Scientific Note: biochemical profile of rats fed yogurt containing yerba mate (Ilex paraguariensis St. Hil) extract. **Brazilian Journal of Food Technology,** v. 14, n. 4, p. 332-337, 2011.

ROHAN, T. E.; MCMICHAEL, A. J. Methylxanthines and breast cancer. **Int J Cancer,** v. 41, n. 3, p. 390-3, Mar 15 1988.

RONCO, A. L. et al. Mate and tea intake, Dietary antioxidants and risk of breast cancer: a case-control study. **Asian Pac J Cancer Prev,** v. 17, n. 6, p. 2923-2933, 2016.

RONCO, A. L. et al. 'Mate' Intake, Hormone-Based Risk Factors and Breast Cancer: a Case-Control Study. **Asian Pac J Cancer Prev,** v. 18, n. 4, p. 941-948, Apr 1 2017.

RONCO, A. L. et al. Mate Intake and Risk of Breast Cancer in Uruguay: a Case-Control Study. **Asian Pac J Cancer Prev,** v. 17, n. 3, p. 1453-61, 2016.

ROSENDAHL, A. H. et al. Caffeine and caffeic acid inhibit growth and modify estrogen receptor (ER) and insulin-like growth factor I receptor (IGF-IR) levels in human breast cancer. **Clinical Cancer Research**, 2015.

RUTTKAY-NEDECKY, B. et al. The role of metallothionein in oxidative stress. **Int J Mol Sci,** v. 14, n. 3, p. 6044-66, Mar 15 2013.

SCHINELLA, G. et al. Antioxidant effects of an aqueous extract of Ilex paraguariensis. **Biochemical and Biophysical Research Communications,** v. 269, n. 2, p. 357-360, 2000.

SCHREIBER-DETURMENY, E.; BRUGUEROLLE, B. Simultaneous high-performance liquid chromatographic determination of caffeine and theophylline for routine drug monitoring in human plasma. **Journal of Chromatography B: Biomedical Sciences and Applications,** v. 677, n. 2, p. 305-312, 1996.

SEN, C. K.; PACKER, L. Antioxidant and redox regulation of gene transcription. **FASEB J,** v. 10, n. 7, p. 709-20, May 1996.

SERRA, K. P. et al. Nova classificação dos carcinomas da mama: procurando o luminal A. **Rev Bras Ginecol Obstet,** v. 36, n. 12, p. 575-580, 2014.

SI, M.; LANG, J. The roles of metallothioneins in carcinogenesis. **J Hematol Oncol,** v. 11, n. 1, p. 107, Aug 23 2018.

STEFANI, E. D. et al. Mate consumption and risk of cancer: a multi-site case-control study in Uruguay. **Asian Pac J Cancer Prev,** v. 12, n. 4, p. 1089-93, 2011.

TRAVERSO, N. et al. Role of glutathione in cancer progression and chemoresistance. **Oxid Med Cell Longev,** v. 2013, p. 972913, 2013.

TURASHVILI, G. et al. Novel immunohistochemical markers for the differentiation of lobular and ductal invasive breast carcinomas. **Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub,** v. 151, n. 1, p. 59-64, Jun 2007.

TWOROGER, S. S. et al. Caffeine, alcohol, smoking, and the risk of incident epithelial ovarian cancer. **Cancer,** v. 112, n. 5, p. 1169-77, Mar 1 2008.

VIEIRA, D. S. C. et al. Carcinoma de mama: novos conceitos na classificação. **Revista Brasileira de Ginecologia e Obstetricia**, 2008.

WARIS, G.; AHSAN, H. Reactive oxygen species: role in the development of cancer and various chronic conditions. **J Carcinog,** v. 5, p. 14, May 11 2006.

WERNER, J. et al. A real-time PCR method for the quantification of the two isoforms of metallothionein in Lake Trout (Salvelinus namaycush). **Arch Environ Contam Toxicol,** v. 54, n. 1, p. 84-91, Jan 2008.

WILHELM FILHO, D. et al. Effect of different oxygen tensions on weight gain, feed conversion, and antioxidant status in piapara, Leporinus elongatus (Valenciennes, 1847). **Aquaculture,** v. 244, n. 1-4, p. 349-357, 2005.

YANG, J.; LIU, R. H. The phenolic profiles and antioxidant activity in different types of tea. **International Journal of Food Science & Technology,** v. 48, n. 1, p. 163-171, 2013.

5.ARTIGO CIENTIFICO

Chimarrão consumption and prognostic factors in breast cancer: Correlation with antioxidants and caffeine blood levels.

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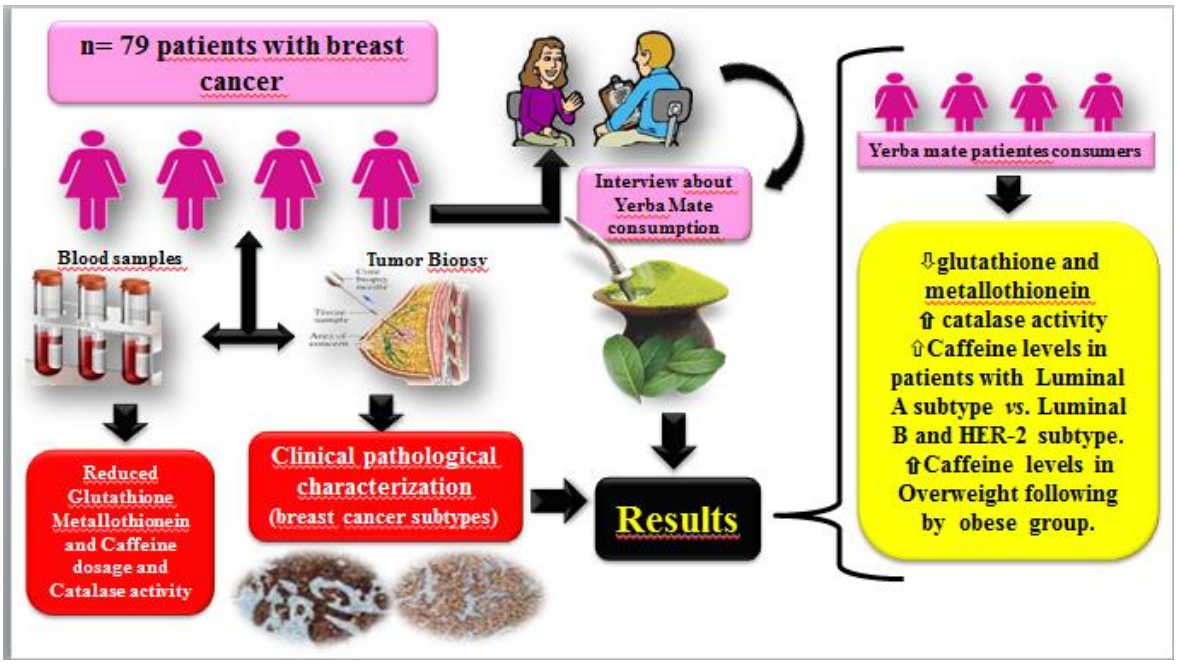
ABSTRACT

Introduction: Breast cancer is the second most common malignant neoplasm among women, presenting high clinical and biological heterogeneity, leading to a complex disease. Both internal and external factors are determinant for the disease prevention and progression. *Ilex paraguariensis A*. St. Hil, a specimen of significant interest in ethnopharmacology, consumed in Southern Brazil mainly in the form of chimarrão, displays a complex chemical composition and is rich in antioxidants potentially exploited in several studies, in addition to acting in the prevention of diseases caused by oxidative stress, such as cancer. This study aims to investigate the clinicopathological profile of breast cancer women that consume chimarrão when compared to non-consumer ones. **Methodology:** A total of 199 women treated at the Francisco Beltrão Cancer Hospital (Ceonc) were volunteers from May 2015 to December 2017 presenting suspected breast cancer lesions. All women with benign breast disease were excluded, totaling a group of 79 women presenting breast cancer who presented all the clinico-pathological data for inclusion in the study. Chimarrão consumption was categorized as positive or negative, the antioxidant profile was evaluated through the determination of reduced glutathione and metallothionein contents and catalase activity, in addition to caffeine blood level. **Results and Discussion:** Decreases in reduced Glutathione and metallothionein levels and increases in catalase activity were observed among chimarrão consumers. The levels of circulating caffeine in patients with molecular Luminal A subtype were higher in comparison to Luminal B and subtype HER-2, whereas concerning the distribution agreement with body mass index (BMI), those overweight presented higher caffeine levels than the obese women compared to eutrophic ones. These findings suggest that chimarrão consumption enhances the antioxidant enzymatic defenses, and that its metabolite caffeine is differentially expressed according to clinicopathological parameters that are determinant of breast cancer prognosis.

Keywords: Ilex paraguariensis; breast cancer; chimarrão, caffeine, antioxidants



1.INTRODUCTION

Breast cancer is the second most common malignant neoplasm among women, corresponding to approximately 28% of new cancer cases each year in Brazil and globally (INCA, 2018). Due to its high clinical, biological and morphological heterogeneity, breast cancer is a complex disease. Tumors with similar histological and clinical profiles present different prognoses and different therapeutic responses (Cirqueira et al., 2011; Vieira et al., 2008). Despite the advances in knowledge concerning tumor biology, it is known that external factors, such as habits, cultural aspects, environmental exposure and stress, are decisive in the course of the disease, both in its prevention development and in carcinogenesis promotion (Araújo da Silva and da Silva Riul, 2011).

Ilex paraguariensis A. St. Hil., popularly known as yerba mate, is widely consumed in South American countries like Uruguay, Argentina, Paraguay and Brazil in the form of chimarrão, a hot infusion drink prepared with dried and chopped leaves, with a bitter flavor, presenting stimulating and antioxidant properties (Boaventura et al., 2015)**.** Chimarrão was evolved from a tea ingested by the Guarani ethnic group as a social drink with a ritualistic role in modern South American societies. A main characteristic of chimarrão consumers is the fact that it is ingested continuously during daily living, with individuals ingesting up to 1 liter every 1 hour. This consumption method allows for a continuous extraction of compounds present in dried leavesand steady absorption throughout the day (Bracesco et al., 2011)**.** This scenario suggests that *Ilex paraguariensis*. St. Hil. is a specimen of great significant in ethnopharmacology, and its consumption merits investigation.

The main components of *Ilex paraguariensis,* are alkaloids (methylxanthines), phenolic compounds (chlorogenic acid) and triterpenic saponins (ursolic and oleanoic acids) (Miranda et al., 2008; Ril et al., 2011). The antioxidant potential of the mate infusion has been widely reported in *in vitro* and *in vivo* studies (Boaventura et al., 2012; Boaventura et al., 2015)**.** The antioxidant activity of this drink is mainly attributed to the high content of methylxanthines alkaloids, particularly caffeine and derivatives (Bastos et al., 2007), while the antioxidant caffeine presents anti-inflammatory and anti-apoptotic activity (Alves

and Bragagnolo, 2002)**.** Phenolic compounds are found in high concentrations, mainly chlorogenic acids, which seem to display antitumor activity and the ability to inhibit carcinogenesis (Miranda et al., 2008; Ril et al., 2011), in addition to antioxidant behavior, acting in the prevention of various degenerative diseases caused by oxidative stress, such as cancer (Gerke, 2016). Saponins, which give the chimarrão drink its bitter taste and creates its foam, represent approximately 10% of the total weight of *Ilex paraguariensis* leaves, with its main representatives being ursolic and oleanoic acids, displaying chemoprotective activity (Puangpraphant et al., 2011)**.** These compounds play a role in inflammatory processes, apoptosis, angiogenesis and metastasis through multiple intra- and extra-cellular targets, in addition to acting as inhibitors for the enzyme aromatase, an important characteristic in breast cancer carcinogenesis (Ronco et al., 2017)**.**

In spite of the fact that *Ilex paraguariensis* is an excellent source of bioactive antitumor and antioxidant substances and anti-inflammatory compound drugs, the literature lacks studies demonstrating the impact of its consumption throughout an individual's life on certain prognosis parametersin patients presenting breast cancer. Chimarrão consumption has been pointed out as inversely associated to the risk of developing this disease (Stefani et al., 2011). Furthermore, its consumption has also been characterized as protective against breast cancer development through several mechanisms involving its main components (De Stefani et al., 2011; Ronco et al., 2017; Ronco et al., 2016a; Ronco et al., 2016b)**.**

Studies assessing whether *Ilex paraguariensis* consumption has a protective role in the risk of developing the disease are available in the literature, but they do not focus on investigating what type of clinical outcome occurs in women with the diagnosis and who consume chimarrão throughout their lives, when compared to non-consumers. Thus, this study aims to investigate the clinicopathological profile of breast cancer among women who consumer chimarrão compared to those who do not, and verify possible clinicopathological correlations between chimarrão consumption, caffeine levels and blood antioxidants.

2. MATERIAL AND METHODS

2.1 STUDY DESIGN

A total of 199 women treated at the Francisco Beltrão Cancer Hospital (Ceonc) were volunteers from May 2015 to December 2017, with suspected breast cancer lesions. After diagnosis confirmation, they were categorized as bearers of breast benign diseases or infiltrating breast ductal carcinoma. All women with benign breast disease were excluded from the study, totaling a group of 79 women with breast cancer who presented all the clinico-pathological data for inclusion in the study. All women signed Free and Informed Consent Forms. This study was approved by the Committee for Ethics in Research with human beings of the State University West of Paraná (CAAE No. 35524814.4.0000.0107.)

Chimarrão consumption was categorized as positive or negative, and, due to the difficulty in establishing a consumption standard in liters per day, blood antioxidant profiles and caffeine levels were assessed as evaluation parameters.

The following parameters were considered for the clinico-pathological characterization of the assessed groups: age at disease diagnosis, weight, height, tumor-node-metastasis (TNM) international classification of breast tumors for staging, hormonal status of the tumors, type of instituted chemotherapy regimen, categorization of patient risk, lymph node status and the presence or absence of menopause. Immunohistochemical staining was performed for tumor subtyping regarding the estrogen receptor (ER), progesterone receptor (PR), Ki-67 index of proliferation Ki-67and human epidermal growth factor 2 receptor (HER2). Based on the immunostaining, the tumors were categorized as Luminal A tumors (ER and/or PR positive), Luminal B (ER and/or PR positive, and/or HER2 positive, and/or Ki-67>14%), Luminal HER (ER and/or PR positive, HER2 positive), HER2 (ER, and PR negative, HER2 positive) and triple-negative (all negative staining, regardless of Ki-67 values Ki-67).

2.2 SAMPLE COLLECTION

Sample collection was performed using heparinized peripheral blood (10 mL) followed by plasma separation after centrifugation (Excelsa® Flex 3400 – Fanem, 4,000 *x g*, 5 minutes). The plasma was stored at -20 ºC until the analyses were performed.

2.3 REDUCED GLUTATHIONE (GSH) EXTRACTION AND DETERMINATIONS

GSH extraction followed the protocol reported by Beutler (1975), with modifications proposed by Wilhelm Filho et al. (2005). Aliquots of 150 µL of each sample were pippeted into sterile polypropylene microtubes (2 mL capacity) and homogenized in a sodium phosphate buffer 0.1 mol L^{-1} solution at pH 6.5, containing sucrose 0.25 mol L^{-1} and EDTA 1 mmol L^{-1}. The samples were then centrifuged in a refrigerated centrifuge (model 5430R, Eppendorf, São Paulo, SP, Brazil) at 11,000 *x g* at 4 ºC for 30 minutes. The final supernatants were then transferred to other microtubes and frozen in an ultra-freezer at -80ºC until analysis. The GSH determination in the supernatants was performed by the spectrophotometric method through Ellman's reaction at 412 nm (Ellman, 1959), using an analytical curve plotted with GSH as the external standard.The results were expressed as wet weight mmol g^{-1} wet weight (ww).

2.4 CATALASE ACTIVITY (CAT) DETERMINATIONS

Catalase activity was assayed according to the method described by Lushchak et al. (2005). The reactional medium was comprised 1.0 mmol L^{-1}EDTA, 20 mmol $L^{-1}H_2O_2$ in a sodium phosphate buffer 0.1 mol L^{-1} solution, pH 7.5. The H_2O_2 consumption was continuously recorded on a spectrophotometer (λ = 240 nm) at 25 ºC. The calculations to determine the enzymatic activity were performed using the H_2O_2 absorptivity coefficient (ε_{240} = 43.6 $mmol^{-1}cm^{-1}$).

2.5 METALLOTHIONEIN (MT) EXTRACTION AND DETERMINATIONS

Metallothioneins were extracted by thermal extraction following the protocol reported by Erk et al. (2002), with adaptations. This process consisted in weighing 150 µL of each sample in sterile polypropylene microtubes (2 mL capacity),

followed by homogenization in a Tris-HCl buffer solution at pH 8.6, containing phenyl-methyl-sulfonyl-fluoride as a protease inhibitor and β- mercaptoethanol as the reducing agent in a vortex mixer for 1 minute. Subsequently, the samples were centrifuged at 20,000 *x g* at 4 ºC during 60 minutes in a refrigerated centrifuge (5430R, Eppendorf, São Paulo, SP, Brazil). After centrifugation, the supernatants were transferred to other microtubes and heated at 70 ºC for 10 minutes. After heating, the samples were centrifuged again at 20,000 x g at 4 ºC for 30 minutes. The final purified supernatants were then transferred to new microtubes and frozen at -80ºC until analysis. MT determinations in the supernatants was performed by a spectrophotometric method by Ellman's reaction at 412 nm (Ellman, 1959), using an analytical curve plotted with GSH as external standard, estimating MT levels as 1 mol MT being the equivalent of 20 mol GSH (Kagi, 1991).

2.6. CAFFEINE BLOOD LEVEL DETERMINATIONS

Caffeine levels were determined by High Performance Liquid Chromatography (HPLC) in the plasma of the collected samples. The system consisted of a Shimadzu HPLC apparatus (Shimadzu Scientific Instruments, Norcross, GA, USA), coupled with an automatic HTC PAL sampler (Leap Technologies, CTC Analytics, Carrboro, USA). The chromatographic separation was carried out using a C18 column (150 mm x 3mm, Phenomenex, Torrance, USA) applying an elution gradient, using a water and methanol mixture as the mobile phase A (95:5, v/v) and the mobile phase B (10:90, v/v), both containing formic acid at 0.05%, as described by Schreiber-Deturmeny and Bruguerolle (1996). The detection was performed using a API MDS Sciex 3000 quadrupole mass spectrometer (Applied Biosystems, Foster City, USA. All analyses were performed in the monitoring mode for multiple reactions. Instrument control and data acquisition were performed using the Analyst software package (Applied Biosystems, Foster City, USA). Sample preparation was performed by protein precipitation with methanol. Aliquots (50 µL) of plasma samples, standards and, quality control samples were transferred to 0.5 mL microcentrifuge tubes and 175 µL of cold methanol containing 10 mL of an internal standard (10 ng mL^{-1}) were

added to each tube. The samples were then briefly mixed using a vortex mixer at high speed, and kept on ice for 40 min and subsequently centrifuged at 14,000 x *g* for 10 min at 4 ° C. Approximately 120 μL of supernatant from each tube were transferred to a clean amber automatic sampler vial, with insert for analysis. A 10 μL aliquot of the solution was then injected into the LC-MS/MS system. The test was then validated for linearity, accuracy, precision and recovery.

2.6 STATISTICAL ANALYSES

All analyses were performed in duplicate, and the data expressed as median (for non-parametric data) or means (for parametric data) ± standard error of the mean. The results were assessed with Grubbs test for the detection of outliers, and no outliers were detected. The results were compared by Student's t test or the Mann-Whitney test, in accordance to the distribution of variances, considering $p<0.05$ as significant. All statistical analyses were performed using the GraphPad Prism 7.0 software package (GraphPad Software, San Diego, CA, USA). The SPSS 22.0 software (IBM, USA) was used to obtain the frequencies and apply the Chi-square test and logistic regression analyses.

3. RESULTS

A total of 79 patients with a positive diagnosis for cancer were included in this study. The detailed data of the clinico-pathological characteristics of the patients involved in the study are displayed in Table 1. The mean age at diagnosis was of 55.29 ± 1.07 years old and 77.2% were aged greater than or equal to 45 years. Regarding menopausal status, 65.8% of the patients had already entered the menopausal period, against 29.1% non-menopausal women. The body mass index (BMI) of the assessed group was of 29.50 ± 0,4Kg/m^2, demonstrating that the most prevalent BMI category was overweight (44.3%), followed by those presenting obesity (regardless of the degree), at 30.4%.

Concerning tumor molecular classification, 24.1% were classified as Luminal A, 36.7% as Luminal B, 21.5% as triple negative, 8.9% as Luminal HER-2, and only 7.6% as HER-2.

Concerning physical tumor characteristics, tumor size varied by up to 2 cm in 49.4% of the patients, while tumors ranging from 2 to 5 cm were present in

29.1% of the patients. The biopsy results indicated that 69.6% of the tumors displayed a low degree of cell differentiation.

The patients' risk stratification was considered high in 54.4% of the samples, followed by low in 30.4%. Of the analyzed patients, 62% presented no compromised lymph nodes, while 8.9% of the cases presented over 5 compromised lymph nodes.

Among the 79 individuals diagnosed with breast cancer, 78.5% (62) reported drinking chimarrão.

Table 1 – Clinico-pathological patient data

Variables	Categories	%	Breast Cancer
Patients (total)		100	79
Age at diagnosis	Up to 45 years old	22.3	16
	Above 45 years old	77.2	61
Menopause	Yes	65.8	52
	No	29.1	23
Body mass index (BMI)	Low weight	1.3	01
	Eutrophic	19.0	15
	Overweight	44.3	35
	Obese	30.4	24
Molecular subtype	Luminal A	24.1	19
	Luminal B	36.7	29
	Luminal HER-2	7.6	06
	HER-2	8.9	07
	Triple negative	21.5	17
Compromised lymph nodes	No commitment	62.0	49
	Up to 5 nodes	20.3	16
	Above 5 nodes	8.9	07
Tumor size	Up to 2 cm	49.4	39
	Between 2-5 cm	29.1	23
	Over 5 cm	12.7	10

Presence of angiolymphatic emboli	No	60.8	48
	Yes	35.4	28
Histological grade	Low	69.6	55
	High	24.1	19
Risk stratification	Low	30.4	24
	Intermediate	7.6	06
	High	54.4	43
Chimarrão (yerba mate) consumption	Yes	78.5	62
	No	17.7	14

Regarding the antioxidant parameter analysis of patients with breast cancer in relation to chimarrão consumption, reduced glutathione (GSH) plasma levels were lower among chimarrão consumers compared to those who do not consume this beverage (0.674 ± 0.02 *vs*. 1.7 ± 0.0021; $p< 0.0001$). These results are displayed in Figure 1. Plasma metallothionein levels in consumers, on the other hand, were reduced in comparison to non-consumers (Figure 2, 0.06 ± 0.0002 mmol g^{-1} ww for non-consumers and 0.03± 0.0002 mmol g^{-1} ww chimarrão consumers, p=0.001).

Concerning serum catalase activity (CAT), increased activity was observed only in chimarrão consumers when compared to the non-consumers (1.01 ± 0.085 *vs*. 0.4577 ± 0.001; p=0.0225). This data is displayed in Figure 3.

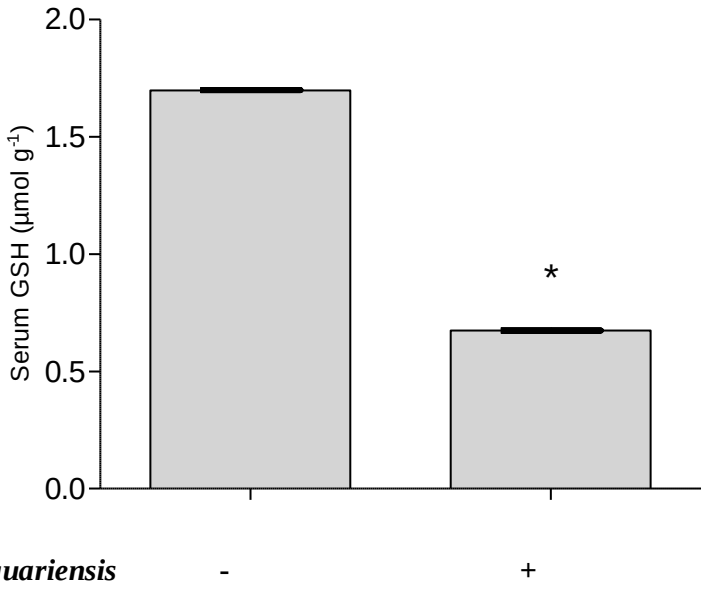


Fig.1- Circulating reduced glutathione levels in the plasma of patients with breast cancer regarding chimarrão consumption. Data are presented as means ± standard error of the means. * indicates a statistically significant difference in relation to chimarrão consumption .

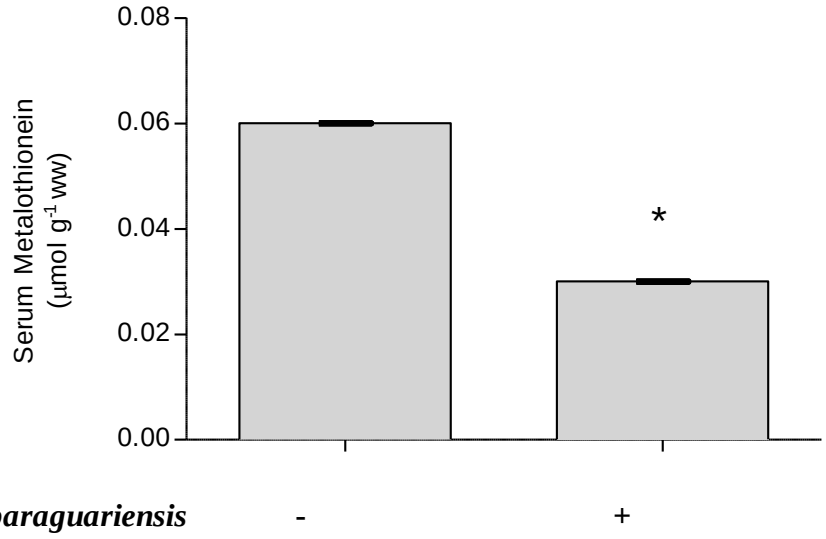


Fig.2- Circulating metallothionein levels in the plasma of patients with breast cancer regarding chimarrão consumption. Data are presented as means ± standard error of the means. * indicates a statistically significant difference in relation to chimarrão consumption .

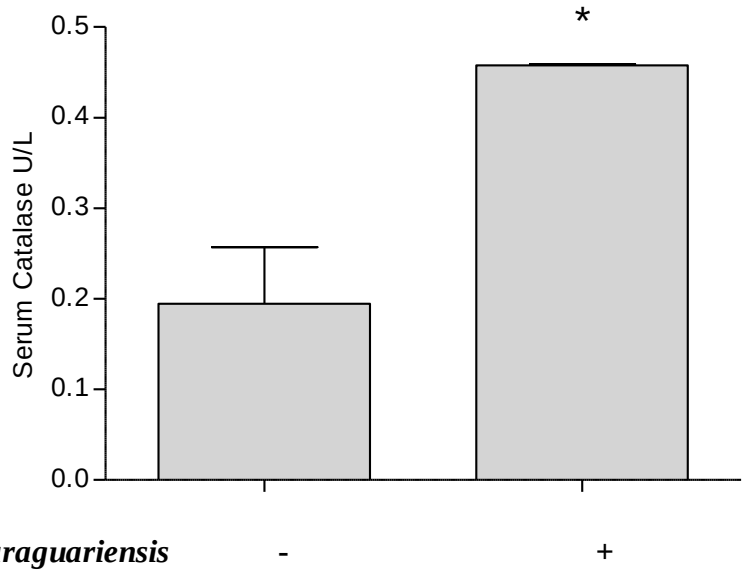


Fig.3- Catalase activity in the plasma of patients with breast cancer regarding chimarrão consumption. Data are presented as means ± standard error of the means. * indicates a statistically significant difference in relation to chimarrão consumption .

Among women with breast cancer who were chimarrão consumers (Table 2), the data suggest a positive association with excess weight (overweight and obese) at diagnosis.

Table 2 - Body mass index (BMI) of women with breast cancer, distributed according to their chimarrão consumption profile.

	Chimarrão		
	No	Yes	Total
BMI			
Low weight	0	1 (100%)	1 (100%)
Eutrophic	3 (20%)	12 (80%)	15 (100%)
Overweight	8 (22.9%)	27 (77.1%)	35 (100%)
Obesity	3 (12.5%)	19 (79.1%)	24 (100%)

*Logistic regression (Chimarrão x BMI overweight), p-value 0.007^{b},R^2 0,78.

Regarding menopause status at diagnosis for women with breast cancer and chimarrão consumers, a significant association between chimarrão consumption and menopause at diagnosis was observed (Table 3).

Table 3 - Distribution of menopausal status at diagnosis of women with breast cancer according to chimarrão consumption.

	Chimarrão		Total
	No	Yes	
Menopause			
No	6 (26.1%)	17 (73.9%)	23 (100%)
Yes	7 (13.5%)	44 (84.6%)	52 (100%)

*Logistic regression (Chimarrão x menopause), p-value 0.001, R^2 0.274.

Caffeine serum levels in patients with breast cancer presented a median of 0.431 μg mL^{-1} (Figure 4). Due to this fact, the possible existence of correlations between caffeine plasma and clinico-pathological parameters was subsequently investigated in the assessed patients.

Plasma caffeine level assessmenst were distributed in accordance with the molecular subtype of the tumors (Figure 5). Caffeine levels were reduced in subtype HER-2 in comparison to the Luminal A Subtype (HER-2: 0.35,±,0.08 μg mL^{-1} vs. Luminal A: 0.82,±,0.12 μg mL^{-1} ; p=0.049) and also reduced in the Luminal B subtype compared to Luminal A (Luminal B: 0.51 ± 0.1 μg mL^{-1} *vs*. Luminal A: 0.82 ± 0.12 μg mL^{-1}; p=0.0083). No statistically significant differences (p>0.05) were noted for the other comparisons among histopathological subtypes.

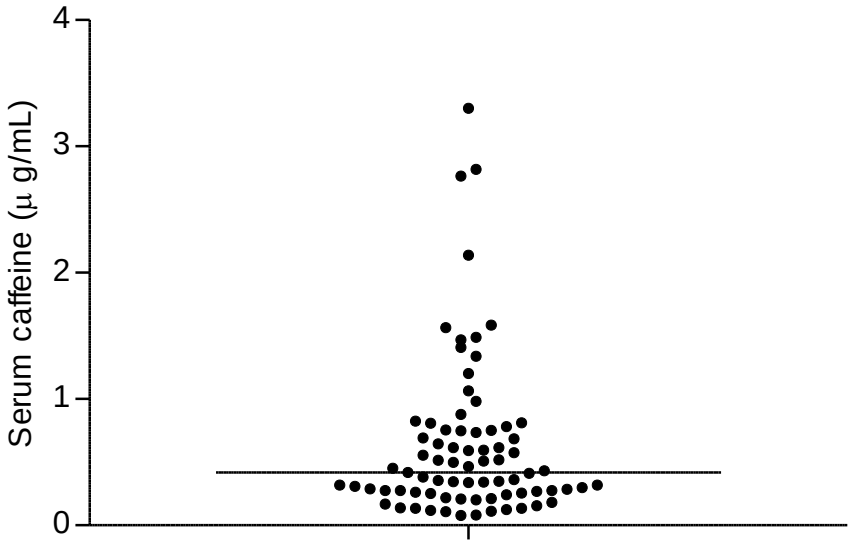


Fig.4 - Serum caffeine levels in breast cancer patients.

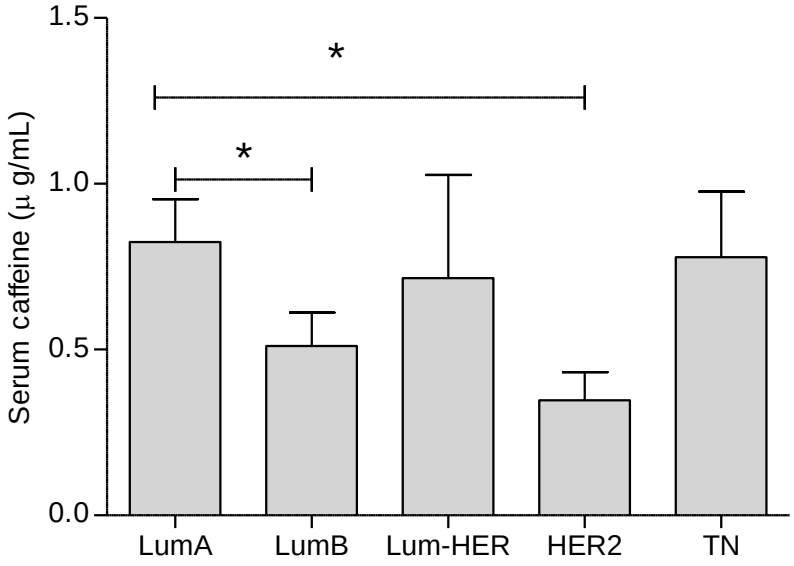


Fig.5- Serum caffeine according to breast cancer subtypes. Data are presented as means ± standard error of the means. * indicates a statistically significant difference**.**

Plasma caffeine levels when distributed according to the BMI in women diagnosed with breast cancer (Figure 6) indicate that those overweight (0.79 ± 0.13 μg mL^{-1} *vs*. 0.36 ± 0.05 μg mL^{-1}; p= 0.0396) presented the highest caffeine

levels, followed by obese individuals (0.58 ± 0.08 μg mL^{-1} *vs*. 0.36 ± 0.05 μg mL^{-1} ; p= 0.048) when compared to eutrophic individuals.

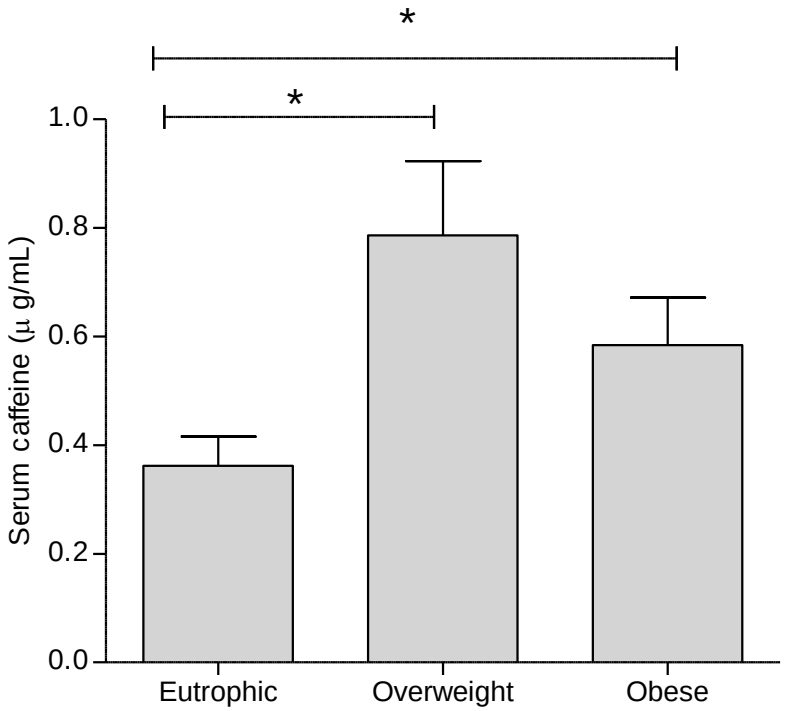


Fig.6- Serum caffeine according to patient body mass index. Data are presented as means ± standard error of the means. * indicates a statistically significant difference.

No correlations were observed with the other clinicopathological parameters evaluated in this study (Table 4)

Table 4- Caffeine plasma levels in the studied group, distributed according to the other assessed clinicopathological parameters.

Clinico-pathological parameters	**Caffeine (means ± standard error, μg mL^{-1})**
Compromised lymph nodes	
Positive	0.69±0.16
Negative	0.69±0.14

Histological grade	
Low	0.56±0.07
High	0.79±0.18
Menopausal status	
Yes	0.62±0.09
No	0.65±0.15

4. DISCUSSION

4.1 – Antioxidant profile of breast cancer patients and yerba mate intake.

The present study suggests that chimarrão consumption decreases reduced glutathione and metallothionein levels while increasing catalase activity in the plasma of patients with breast cancer and *Ilex paraguariensis* consumers in comparison to non-consumers. It is important to highlight that this is the first study investigating the blood antioxidant profile of patients with breast cancer and conducting comparisons between *Ilex paraguariensis* consumers and non-consumers.

It is known that oxidative stress is the pathogenic outcome resulting from an imbalance between the production of reactive oxygen species (ROS) and the antioxidant capacity of cells (Landriscina et al., 2009)**.** In addition**,** in breast cancer cases, this is a very well established and associated condition with several important clinical outcomes (Crujeiras et al., 2013; Panis et al., 2012b)**.** In this context, the evaluation of the blood antioxidant profile, and its relationship with chimarrão consumption in these patients, may contribute to the understanding of the role of the continued consumption of food antioxidants in patients presenting this disease.

The consumption of *Ilex paraguariensis* by healthy individuals leads to increased GSH levels when compared to baseline values prior to its consumption (Boaventura et al., 2015). Yerba mate contains several bioactive compounds rich in redox modulating properties, which act against ROS (Colpo et al., 2017), which may justify its effect in healthy individuals. This has also been observed in patients

presenting chronic diseases, such as diabetes mellitus type II (Boaventura et al., 2013), dyslipidemia and cardiovascular risk (Boaventura et al., 2012). However, our results indicate that chimarrão consumption in patients with breast cancer does not promote this increase. Patients with breast cancer often present reduced levels of antioxidants (Panis et al., 2012a) and the results reported herein suggest that chimarrão consumption is not capable of reverting this GSH depletion, but, in fact, enhances its reduction. These findings suggest that chimarrão consumption in patients with breast cancer can regulate GSH synthesis, perhaps by providing high levels of exogenous antioxidants through its intake. The same profile was noted for metallothionein.

Metallothioneins belong to a group of low molecular weight proteins rich in cysteines (30%). In humans, one of the 11 MT isoforms deserves special attention, namely metallothionein 2A (MT2A), expressed in many organs and tissues, and involved in the regulation of metal homeostasis, detoxification, oxidative stress and immune defenses, cell cycle progression, cell proliferation and differentiation and angiogenesis (Ling et al., 2016). Some studies have demonstrated that MT2A is a ROS scavenger, acting in cell and tissue protection against oxidative stress (Baird et al., 2006; Qu et al., 2013; Yang and Chitambar, 2008)**.** MT2A act as a powerful zinc chelator when excessive amounts of this metal are present, and as a ROS eliminators when increased oxidative stress conditions occur (Xue et al., 2009). It is important to emphasize that MT2A may also regulate mitogen-activated kinase proteins (MAPKs) and play an important role in anti-oxidant, anti-apoptotic and anti-inflammatory responses (Ling et al., 2016; Ruttkay-Nedecky et al., 2013)**.**

It is known that MT serum levels are positively correlated to the pathological condition, disease stage and degree of cancer progression. MT play an important active and critical role in the carcinogenesis process, namely in tumor growth and progression (positively correlated with Ki-67), metastasis (MT may induce positive gene adjustment related to angiogenesis), and resistance to antitumor therapies (Si and Lang, 2018)**.** Thus, increased MT expression induces anti-apoptotic activity in tumor cells (interacting with NF-KB, and inactivating p53), are also related to resistance to multiple anti-tumor drugs (Phenomenon resistance to multiple drugs - MDR), and increased concentrations inside the cell are directly

related to the degree of tumor malignancy, thus becoming a potential prognostic marker (Bizon et al., 2017)**.**

Studies involving MT expression and chimarrão consumption are not available in the literature. However, one study demonstrated a positive correlation between the antioxidant activity of flavonoids (one of the main classes of biocompounds found in yerba mate) and MT expression (Weng et al., 2011). In another study concerning flavonoids, an induction, in a dose dependent manner, of the expression of the RNA messenger of MT2 alongside other isoforms was reported (Chung et al., 2006). A study on intestinal cancer cells (Caco2) also reported RNA messenger expression induction of MT, evidencing the effect of flavonoids on the expression of this protein (Kameoka et al., 1999). However, the results reported herein indicate that patients with breast cancer and chimarrão consumers do not present high MT levels, but instead, reduced levels, suggesting that chimarrão consumption could negatively regulate the expression of this protein.

An antioxidant enzyme investigated herein was catalase, which metabolizes hydrogen peroxide (H_2O_2) into water and oxygen, also decomposes peroxynitrite and can oxidize nitric oxide into nitrogen dioxide (Glorieux et al., 2014)**.** Levels of antioxidant enzymes, such as catalase, are reduced in a variety of lineage of tumor cells when compared to normal cells, suggesting that hydrogen peroxide is not effectively removed in these cells (Nishikawa, 2008) **.** Catalase overexpression in breast tumor cells have been reported as decreasing the capacity for cell proliferation and migration, contributing in improving responses to antitumor treatment (Glorieux et al., 2011). Another study has suggested that the accumulation of hydrogen peroxide as a result of decreased catalase activity is correlated with tumor metastasis (Tsai et al., 2014)**,** accompanied by an increase in the growth rate of cancerous cells, and that decreased hydrogen peroxide levels regulate PP2A activity, inducing cellular apoptosis (Sen et al., 2012)**.**

The results of the present study indicate that catalase activity was increased in *Ilex paraguariensis* consumers compared to non-consumers, suggesting that chimarrão consumption by patients suffering from breast cancer positively modulates catalase activity. Boaventura et al. (2015) observed that the

consumption of *Ilex paraguariensis,* by 31 healthy people before and after an hour increases catalase activity in 30% demonstrating a significant positive association. In another study, Matsumoto et al. (2009) observed a significant increase in catalase activity after 15 days of *Ilex paraguariensis* consumption, proposing that the bioactive compounds present in yerba mate or their metabolites may mediate this enzyme.

4.3 Caffeine levels in patients with breast cancer according to their clinico-pathological classification

In addition to the evaluation of antioxidant blood levels in patients with breast cancer who consume chimarrão, another aim of this study was to establish a correlation between caffeine circulating levels and the clinicopathological parameters of the disease. In addition, due to the continued consumption of chimarrão reported by the assessed patients, the study of caffeine blood levels aided in the understanding of how this substance could reflect the systemic impact of the ingestion of this beverage.

Caffeine is an alkaloid belonging to the methylxanthines group, and one of the main active compounds in yerba mate (Bastos et al., 2007). Caffeine has its mechanism of action associated with antitumor mechanisms, such as cell cycle suppression and proliferation through the induction of p53, in addition to inducing apoptosis (Bode and Dong, 2007)**.** It is positively correlated with levels of globulin bound to sex hormones and with lower levels of bioavailable estrogens in post and pre-menopausal women (Ferrini and Barrett-Connor, 1996; Kotsopoulos et al., 2009)**.** In this study, higher circulating caffeine levels were detected in patients presenting the Luminal A subtype, a hormone-dependent tumor. Thus, the caffeine levels found in these patients suggest a higher consumption of chimarrão, which can contribute to a better prognosis, since this tumor subtype is dependent on estrogen and caffeine tends to make this hormone less bioavailable.

In relation to caffeine levels distributed according to BMI, women with breast cancer who were overweight reported higher chimarrão consumption (Table.2). No studies are available assessing caffeine plasma levels in this context, which hindered a more accurate exploration of the study observations. In addition, no reports on the consumption of caffeinated beverages in breast cancer

patients and their associations with BMI have been published. The high levels of plasma caffeine observed herein suggest a higher consumption of yerba mate in the form of chimarrão by women with excess weight. Chimarrão has shown a positive effect on weight reduction, which may have influenced the higher consumption by these women who are seeking an possible weight loss effect (Jung and Hur, 2016).

Obesity is associated with multiple circulating factors that can operate independently, as well as in conjunction, with estrogen in influencing the development of breast cancer (Cleary and Grossmann, 2009). Luminal cancers (estrogen-dependent) present a positive connection with excess weight, and these tumors may be largely mediated by increased estrogen levels related to excessive adiposity (Arnold et al., 2016). Adipose tissue expresses increased activity of the aromatase enzyme, which leads to increases in circulating estrogens mainly in post-menopausal women, leading to increased cellular differentiation and proliferation, in addition to apoptosis inhibition (Calle and Kaaks, 2004).

5. CONCLUSIONS

This study indicated that *Ilex paraguariensis* consumption in the form of chimarrão is able to promote antioxidant improvements only in relation to catalase activity, reducing reduced glutathione and metallothionein levels in the plasma of the cohort of patients with breast cancer assessed herein. Chimarrão consumption was associated to excess weight (overweight and obesity independent of degree) and menopause at diagnosis, these observations are peculiar of the cohort of patients in this study, and plasma caffeine levels were increased in patients with Luminal A molecular subtype tumors in comparison to the Luminal B subtype and HER-2. The distribution of caffeine serum levels according to BMI demonstrated that overweight individuals displayed higher caffeine levels followed by obese individuals when compared to eutrophic patients in each chimarrão consumption subgroup. It is important to highlight that this is the first study to investigate the antioxidant profile of patients with breast cancer and chimarrão consumers, quantifying caffeine serum concentrations and correlating them with clinicopathological factors. These results provide additional evidence to support

the role of yerba mate and caffeine in carcinogenesis progression and prevention, as well as better response to treatment and improvement of clinical-pathological parameters in breast cancer cases.

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7. REFERENCES

Alves, A.B. and Bragagnolo, N., 2002. Determinação simultânea de teobromina, teofilina e cafeína em chás por cromatografia líquida de alta eficiência. Revista Brasileira de Ciências Farmacêuticas 38, 237-243.

Araújo da Silva, P. and da Silva Riul, S., 2011. Câncer de mama: fatores de risco e detecção precoce. Revista Brasileira de Enfermagem 64.

Arnold, M., Leitzmann, M., Freisling, H., Bray, F., Romieu, I., Renehan, A. and Soerjomataram, I., 2016. Obesity and cancer: an update of the global impact. Cancer epidemiology 41, 8-15.

Baird, S.K., Kurz, T. and Brunk, U.T., 2006. Metallothionein protects against oxidative stress-induced lysosomal destabilization. Biochem J 394, 275-283.

Bastos, D.H., Saldanha, L.A., Catharino, R.R., Sawaya, A.C., Cunha, I.B., Carvalho, P.O. and Eberlin, M.N., 2007. Phenolic antioxidants identified by ESI-MS from Yerba mate (Ilex paraguariensis) and green tea (Camelia sinensis) extracts. Molecules 12, 423-432.

Beutler, E., 1975. Red cell metabolism: a manual of biochemical methods. Grune & Stratton.

Bizon, A., Jedryczko, K. and Milnerowicz, H., 2017. The role of metallothionein in oncogenesis and cancer treatment. Postepy Hig Med Dosw (Online) 71, 98-109.

Boaventura, B.C., Di Pietro, P.F., Stefanuto, A., Klein, G.A., de Morais, E.C., de Andrade, F., Wazlawik, E. and da Silva, E.L., 2012. Association of mate tea (Ilex paraguariensis) intake and dietary intervention and effects on oxidative stress biomarkers of dyslipidemic subjects. Nutrition 28, 657-664.

Boaventura, B.C.B., da Silva, E.L., Liu, R.H., Prudêncio, E.S., Di Pietro, P.F., Becker, A.M. and Amboni, R.D.d.M.C., 2015. Effect of yerba mate (Ilex paraguariensis A. St. Hil.) infusion obtained by freeze concentration technology on antioxidant status of healthy individuals. LWT-Food Science and Technology 62, 948-954.

Boaventura, B.C.B., Di Pietro, P.F., Klein, G.A., Stefanuto, A., de Morais, E.C., de Andrade, F., Wazlawik, E. and da Silva, E.L., 2013. Antioxidant potential of mate tea (Ilex paraguariensis) in type 2 diabetic mellitus and pre-diabetic individuals. Journal of Functional Foods 5, 1057-1064.

Bode, A.M. and Dong, Z., 2007. The enigmatic effects of caffeine in cell cycle and cancer. Cancer Lett 247, 26-39.

Bracesco, N., Sanchez, A.G., Contreras, V., Menini, T. and Gugliucci, A., 2011. Recent advances on Ilex paraguariensis research: minireview. J Ethnopharmacol 136, 378-384.

Calle, E.E. and Kaaks, R., 2004. Overweight, obesity and cancer: epidemiological evidence and proposed mechanisms. Nat Rev Cancer 4, 579-591.

Chung, M.J., Kang, A.-Y., Lee, K.M., Oh, E., Jun, H.-J., Kim, S.-Y., Auh, J.H., Moon, T.-W., Lee, S.-J. and Park, K.-H., 2006. Water-soluble genistin glycoside isoflavones up-regulate antioxidant metallothionein expression and scavenge free radicals. Journal of agricultural and food chemistry 54, 3819-3826.

Cirqueira, M.B., Moreira, M.A.R., Soares, L.R. and Freitas-Júnior, R., 2011. Subtipos moleculares do câncer de mama. Femina 39.

Cleary, M.P. and Grossmann, M.E., 2009. Obesity and breast cancer: the estrogen connection. Endocrinology 150, 2537-2542.

Colpo, A.C., de Lima, M.E., Maya-López, M., Rosa, H., Márquez-Curiel, C., Galván-Arzate, S., Santamaría, A. and Folmer, V., 2017. Compounds from Ilex paraguariensis extracts have antioxidant effects in the brains of rats subjected to chronic immobilization stress. Applied Physiology, Nutrition, and Metabolism 42, 1172-1178.

Crujeiras, A.B., Diaz-Lagares, A., Carreira, M.C., Amil, M. and Casanueva, F.F., 2013. Oxidative stress associated to dysfunctional adipose tissue: a potential link between obesity, type 2 diabetes mellitus and breast cancer. Free Radic Res 47, 243-256.

De Stefani, E., Moore, M., Aune, D., Deneo-Pellegrini, H., Ronco, A.L., Boffetta, P., Correa, P., Acosta, G., Mendilaharsu, M. and Luaces, M., 2011. Maté consumption and risk of cancer: a multi-site case-control study in Uruguay. Asian Pac J Cancer Prev 12, 1089-1093.

Ellman, G.L., 1959. Tissue sulfhydryl groups. Arch Biochem Biophys 82, 70-77.

Erk, M., Ivanković, D., Raspor, B. and Pavičić, J., 2002. Evaluation of different purification procedures for the electrochemical quantification of mussel metallothioneins. Talanta 57, 1211-1218.

Ferrini, R.L. and Barrett-Connor, E., 1996. Caffeine intake and endogenous sex steroid levels in postmenopausal women. The Rancho Bernardo Study. Am J Epidemiol 144, 642-644.

Gerke, I.B.B., 2016. Extrato aquoso de erva-mate (Ilex paraguariensis St. Hill) clarificado: extração em batelada e processamento por membranas.

Glorieux, C., Auquier, J., Dejeans, N., Sid, B., Demoulin, J.B., Bertrand, L., Verrax, J. and Calderon, P.B., 2014. Catalase expression in MCF-7 breast cancer cells is mainly controlled by PI3K/Akt/mTor signaling pathway. Biochem Pharmacol 89, 217-223.

Glorieux, C., Dejeans, N., Sid, B., Beck, R., Calderon, P.B. and Verrax, J., 2011. Catalase overexpression in mammary cancer cells leads to a less aggressive phenotype and an altered response to chemotherapy. Biochem Pharmacol 82, 1384-1390.

INCA, 2018. Estimativa 2018. Incidência de câncer no Brasil [Internet]. Instituto Nacional de Câncer José Alencar Gomes da Silva. 2017. 130 p.

Jung, J.-H. and Hur, Y.-I., 2016. The Effect of Maté Extract on Body Weight and Fat Reduction in Obese Women: A Randomized Placebo-controlled Clinical Trial. The Korean Journal of Obesity 25, 197-206.

Kagi, J.H., 1991. Overview of metallothionein. Methods Enzymol 205, 613-626.

Kameoka, S., Leavitt, P., Chang, C. and Kuo, S.M., 1999. Expression of antioxidant proteins in human intestinal Caco-2 cells treated with dietary flavonoids. Cancer Lett 146, 161-167.

Kotsopoulos, J., Eliassen, A.H., Missmer, S.A., Hankinson, S.E. and Tworoger, S.S., 2009. Relationship between caffeine intake and plasma sex hormone concentrations in premenopausal and postmenopausal women. Cancer 115, 2765-2774.

Landriscina, M., Maddalena, F., Laudiero, G. and Esposito, F., 2009. Adaptation to oxidative stress, chemoresistance, and cell survival. Antioxid Redox Signal 11, 2701-2716.

Ling, X.-B., Wei, H.-W., Wang, J., Kong, Y.-Q., Wu, Y.-Y., Guo, J.-L., Li, T.-F. and Li, J.-K., 2016. Mammalian metallothionein-2A and oxidative stress. International Journal of Molecular Sciences 17, 1483.

Lushchak, V.I., Bagnyukova, T.V., Lushchak, O.V., Storey, J.M. and Storey, K.B., 2005. Hypoxia and recovery perturb free radical processes and antioxidant potential in common carp (Cyprinus carpio) tissues. Int J Biochem Cell Biol 37, 1319-1330.

Matsumoto, R.L., Bastos, D.H., Mendonca, S., Nunes, V.S., Bartchewsky, W., Ribeiro, M.L. and de Oliveira Carvalho, P., 2009. Effects of mate tea (Ilex paraguariensis) ingestion on mRNA expression of antioxidant enzymes, lipid peroxidation, and total antioxidant status in healthy young women. J Agric Food Chem 57, 1775-1780.

Miranda, D.D., Arcari, D.P., Pedrazzoli, J., Jr., Carvalho Pde, O., Cerutti, S.M., Bastos, D.H. and Ribeiro, M.L., 2008. Protective effects of mate tea (Ilex paraguariensis) on H2O2-induced DNA damage and DNA repair in mice. Mutagenesis 23, 261-265.

Nishikawa, M., 2008. Reactive oxygen species in tumor metastasis. Cancer Lett 266, 53-59.

Panis, C., Herrera, A.C., Victorino, V.J., Campos, F.C., Freitas, L.F., De Rossi, T., Colado Simao, A.N., Cecchini, A.L. and Cecchini, R., 2012a. Oxidative stress and hematological profiles of advanced breast cancer patients subjected to paclitaxel or doxorubicin chemotherapy. Breast Cancer Res Treat 133, 89-97.

Panis, C., Victorino, V.J., Herrera, A.C., Freitas, L.F., De Rossi, T., Campos, F.C., Simao, A.N., Barbosa, D.S., Pinge-Filho, P., Cecchini, R. and Cecchini, A.L., 2012b. Differential oxidative status and immune characterization of the early and advanced stages of human breast cancer. Breast Cancer Res Treat 133, 881-888.

Puangpraphant, S., Berhow, M.A. and de Mejia, E.G., 2011. Mate (Ilex paraguariensis St. Hilaire) saponins induce caspase-3-dependent apoptosis in human colon cancer cells in vitro. Food Chemistry 125, 1171-1178.

Qu, W., Pi, J. and Waalkes, M.P., 2013. Metallothionein blocks oxidative DNA damage in vitro. Arch Toxicol 87, 311-321.

Ril, F.T., Loch, C.R., Valduga, A.T., Macedo, S.M.D. and Cichoski, A.J., 2011. Scientific Note: biochemical profile of rats fed yogurt containing yerba mate (Ilex paraguariensis St. Hil) extract. Brazilian Journal of Food Technology 14, 332-337.

Ronco, A.L., Espinosa, E., Calderon, J.M., Lasalvia- Galante, E., De Rosa, A. and Sanchez, G., 2017. 'Mate' Intake, Hormone-Based Risk Factors and Breast Cancer: a Case-Control Study. Asian Pac J Cancer Prev 18, 941-948.

Ronco, A.L., Stefani, E.D., Mendoza, B., Deneo-Pellegrini, H., Vazquez, A. and Abbona, E., 2016a. Mate Intake and Risk of Breast Cancer in Uruguay: a Case- Control Study. Asian Pac J Cancer Prev 17, 1453-1461.

Ronco, A.L., Stefani, E.D., Mendoza, B., Vazquez, A., Abbona, E., Sanchez, G. and Rosa, A.D., 2016b. Mate and Tea Intake, Dietary Antioxidants and Risk of Breast Cancer: a Case-Control Study. Asian Pac J Cancer Prev 17, 2923-2933.

Ruttkay-Nedecky, B., Nejdl, L., Gumulec, J., Zitka, O., Masarik, M., Eckschlager, T., Stiborova, M., Adam, V. and Kizek, R., 2013. The role of metallothionein in oxidative stress. Int J Mol Sci 14, 6044-6066.

Schreiber-Deturmeny, E. and Bruguerolle, B., 1996. Simultaneous high-performance liquid chromatographic determination of caffeine and theophylline for routine drug monitoring in human plasma. Journal of Chromatography B: Biomedical Sciences and Applications 677, 305-312.

Sen, S., Kawahara, B. and Chaudhuri, G., 2012. Maintenance of higher H(2)O(2) levels, and its mechanism of action to induce growth in breast cancer cells: important roles of bioactive catalase and PP2A. Free Radic Biol Med 53, 1541-1551.

Si, M. and Lang, J., 2018. The roles of metallothioneins in carcinogenesis. J Hematol Oncol 11, 107.

Stefani, E.D., Moore, M., Aune, D., Deneo-Pellegrini, H., Ronco, A.L., Boffetta, P., Correa, P., Acosta, G., Mendilaharsu, M., Luaces, M.E., Silva, C. and Lando, G., 2011. Mate consumption and risk of cancer: a multi-site case-control study in Uruguay. Asian Pac J Cancer Prev 12, 1089-1093.

Tsai, J.Y., Lee, M.J., Dah-Tsyr Chang, M. and Huang, H., 2014. The effect of catalase on migration and invasion of lung cancer cells by regulating the activities of cathepsin S, L, and K. Exp Cell Res 323, 28-40.

Vieira, D.S.C., Dufloth, R.M., Schmitt, F.C.L. and Zeferino, L.C., 2008. Carcinoma de mama: novos conceitos na classificação. Revista Brasileira de Ginecologia e Obstetricia.

Weng, C.-J., Chen, M.-J., Yeh, C.-T. and Yen, G.-C., 2011. Hepatoprotection of quercetin against oxidative stress by induction of metallothionein expression through activating MAPK and PI3K pathways and enhancing Nrf2 DNA-binding activity. New biotechnology 28, 767-777.

Wilhelm Filho, D., Torres, M., Zaniboni-Filho, E. and Pedrosa, R., 2005. Effect of different oxygen tensions on weight gain, feed conversion, and antioxidant status in piapara, Leporinus elongatus (Valenciennes, 1847). Aquaculture 244, 349-357.

Xue, W., Liu, Q., Cai, L., Wang, Z. and Feng, W., 2009. Stable overexpression of human metallothionein-IIA in a heart-derived cell line confers oxidative protection. Toxicol Lett 188, 70-76.

Yang, M. and Chitambar, C.R., 2008. Role of oxidative stress in the induction of metallothionein-2A and heme oxygenase-1 gene expression by the antineoplastic agent gallium nitrate in human lymphoma cells. Free Radic Biol Med 45, 763-772.

Authors contributions

Maria Rachel Pedrazzoli Calixto contributed with sample collection, data analysis and and manuscript ellaboration.

Daniel Rech contributed with patients selection and manuscript ellaboration.

Vanessa Leal dos Santos contributed with sample collection, patient interviews and data obtention from medical records.

Tiago Madeira and Suzana Lucy Nixdorf contributed with caffeine measurement and data analysis.

Tatiane Fagundes, Rachel Ann Hauser Davis, Frederico Freire Bastos, Jayme da Cunha Bastos Neto, Vera Lucia Freire da Cunha Bastos, Ana Carolina Zanandrea and Josivan Ribeiro de Lima, contributed with antioxidants measurement, English revision and manuscript ellaboration.

Vanessa Jacob Victorino and and Carolina Panis were responsible for the design of the study, data analysis and manuscript ellaboration.

6. ANEXOS

JOURNAL OF FUNCTIONAL FOODS

AUTHOR INFORMATION PACK

TABLE OF CONTENTS

- Description p.1
- Audience p.1
- Impact Factor p.1
- Abstracting and Indexing p.2
- Editorial Board p.2
- Guide for Authors p.4

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DESCRIPTION

The *Journal of Functional Foods* aims to bring together the results of fundamental and applied research into healthy foods and biologically active food ingredients.
The Journal is centered in the specific area at the boundaries among food technology, nutrition and health welcoming papers having a good interdisciplinary approach.The journal will cover the fields of plant bioactives; dietary fibre, probiotics; functional lipids; bioactive peptides; vitamins, minerals and botanicals and other dietary supplements. Nutritional and technological aspects related to the development of functional foods and beverages are of core interest to the journal. Experimental works dealing with food digestion, bioavailability of food bioactives and on the mechanisms by which foods and their components are able to modulate physiological parameters connected with disease prevention are of particular interest as well as those dealing with personalized nutrition and nutritional needs in pathological subjects.

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TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO - TCLE

Título do Projeto: Mapeamento do câncer de mama familial no Sudoeste do Paraná e estudo de associação de risco com a exposição ocupacional à agrotóxicos.

Pesquisador responsável: Prof[a] Dr[a] CAROLINA PANIS – Telefones (43)99165316 e (46) 30571079

Convidamos você a participar de nossa pesquisa que tem o objetivo de identificar os casos de câncer de mama em mulheres que tem história da doença na família, que moram na região Sudoeste do Paraná. Para isso será realizada a coleta de um tubo de sangue (10 mL) e um tubo de saliva (1 mL) para fazer os exames necessários para identificar porque alguns tumores de mama levam à doenças tão agressivas.

Durante a execução do projeto também vamos precisar de uma parte do tecido tumoral que o médico irá remover durante a sua cirurgia ou que foi coletado para o diagnóstico da doença (na biópsia). Também precisaremos consultar o prontuário médico, para saber informações sobre sua saúde e sua ocupação de trabalho. Para algum questionamento, dúvida ou relato de algum acontecimento os pesquisadores poderão ser contatados a qualquer momento, pelos telefones (43)99165316 e (46) 30553026. Estamos disponíveis para esclarecer quaisquer dúvidas, a qualquer momento.

Desta forma, você está contribuindo para a identificação de fatores que levam à alta incidência de cânceres agressivos na nossa região.

Este termo será entregue em duas vias, sendo que uma ficará com você. Você não pagará nem receberá para participar do estudo. Seus dados serão mantidos em sigilo, ou seja, ninguém além dos pesquisadores terá acesso ao material ou informações coletadas. Estes dados serão utilizados somente para fins científicos. Você poderá cancelar sua participação a qualquer momento. Se necessitar de maiores informações, o telefone do comitê de ética é 3220-3272 e da pesquisadora responsável é 46 30553026. A coleta de material será feita dentro do Ceonc, portanto qualquer imprevisto será resolvido imediatamente no local. Ao término do projeto, se a pesquisa identificar que a sua doença se classifica como câncer familial, você será chamado ao Ceonc para receber esclarecimentos sobre como proceder no acompanhamento da doença nos próximos anos.

Declaro estar ciente do exposto e desejo participar do projeto.

Nome do sujeito de pesquisa ou responsável:

Assinatura:
CPF:

Eu, _____, declaro que forneci todas as informações do projeto ao participante e/ou responsável.

Data:

Instrumento de coleta de dados histopatológico

Número de identificação (caderno amarelo)	
Nome paciente	
Prontuário	
Data DIAGNOSTICO	
Topografia do câncer	
Idade ao diagnóstico	
Menopausa (sim ou não)	
Finalidade da QT (paliativa, adjuvante, neo)	
Histórico familial (pais ou irmãos com ca)	
Grau histopatológico do tumor	
Dados de Imunohistoquímica	
Classificação TNM	
Invasão Linfonodal	
Invasão vascular	
Fez cirurgia (qual)	
Esquema de drogas da quimio	
Fez radioterapia?	
Tamanho do tumor	
Comorbidades	
Doença recorrente ou primária?	
Sítios de metástase	
Histórico de estresse emocional crônico?	
Histórico médico	
Toma café ou chimarrão? Com que frequência?	
Índice de massa corporal (peso e altura)	
Trabalha Com agrotóxicos	

Sobre a história de ca familial: 1. Algum parente teve ca de ovário? ( ) sim ( ) não 2. Alguém teve ca de mama bilateral? ( ) sim ( ) não 3. Algum homem teve ca de mama? ( ) sim ( ) não 4. Alguém teve ca de ovário E de mama? ( ) sim ( ) não 5. Alguma mulher da família teve ca de mama antes dos 50 anos? ( ) sim ( ) não	

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Mapeamento do câncer de mama familial no sudoeste do Paraná e estudo de associação de risco coma exposição ocupacional à agrotóxicos.

Pesquisador: CAROLINA PANIS

Área Temática:

Versão: 1

CAAE: 35524814.4.0000.0107

Instituição Proponente: UNIVERSIDADE ESTADUAL DO OESTE DO PARANA

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 810.501

Data da Relatoria: 25/09/2014

Apresentação do Projeto:

Neste estudo pretende-se avaliar todas as mulheres diagnosticadas com câncer de mama, atendidas no Hospital de Câncer de Francisco Beltrão (Ceonc), em um período de 48 meses. A partir da análise de anotações em prontuários serão selecionadas para investigação dos genes de interesse aquelas mulheres com história de câncer de mama familial com ou sem exposição ocupacional à agrotóxicos. Atende aos requisitos teóricos, metodológicos e éticos.

Objetivo da Pesquisa:

Mapear os casos de câncer de mama familial na região Sudoeste do Paraná e identificar possível associação a exposição ocupacional à agrotóxicos.

Avaliação dos Riscos e Benefícios:

Não há riscos diretos aos sujeitos, uma vez que serão estudados materiais coletados durante cirurgias oncológicas.

Comentários e Considerações sobre a Pesquisa:

Relevante para a área de oncologia.

Considerações sobre os Termos de apresentação obrigatória:

Todos apresentados.

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UF: PR **Município:** CASCAVEL
Telefone: (45)3220-3272 **E-mail:** cep.prppg@unioeste.br

Recomendações:

Não há recomendações.

Conclusões ou Pendências e Lista de Inadequações:

Não há pendências.

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

Considerações Finais a critério do CEP:

Aprovado. O projeto não necessita adequações.

CASCAVEL, 29 de Setembro de 2014

Assinado por:
João Fernando Christofoletti
(Coordenador)