UNIVERSIDADE DE CRUZ ALTA
UNIVERSIDADE REGIONAL DO NOROESTE DO ESTADO DO RIO GRANDE DO SUL

PROGRAMA DE PÓS-GRADUAÇÃO *STRICTO SENSU* EM ATENÇÃO INTEGRAL À SAÚDE

BIOMARCADORES DA ATEROSCLEROSE SUBCLÍNICA E PRODUTOS NATURAIS COMO MEDICINA ALTERNATIVA E COMPLEMENTAR

DISSERTAÇÃO DE MESTRADO

AMANDA SPRING DE ALMEIDA

CRUZ ALTA – RS, 2016

BIOMARCADORES DA ATEROSCLEROSE SUBCLÍNICA

E PRODUTOS NATURAIS COMO MEDICINA

ALTERNATIVA E COMPLEMENTAR

Por

AMANDA SPRING DE ALMEIDA

Dissertação apresentada ao Programa de Pós-Graduação em Atenção Integral à Saúde, da Universidade de Cruz Alta (UNICRUZ, RS), em associação ampla à Universidade Regional do Noroeste do Estado do Rio Grande do Sul (UNIJUI, RS), como requisito parcial para obtenção de grau de **Mestre em Atenção Integral à Saúde**

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CRUZ ALTA -RS, Brasil

2016

A447b Almeida, Amanda Spring de

Biomarcadores da aterosclerose subclínica e produtos naturais como medicina alternativa e complementar/ Amanda Spring de Almeida. – 2016.

47 f.

Dissertação (mestrado) – Universidade de Cruz Alta - UNICRUZ/RS. Universidade Regional do Noroeste do Estado do Rio Grande do Sul - UNIJUI/RS, Programa de Pós-Graduação em Atenção Integral à Saúde.

Mestrado em atenção integral à saúde.

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Coorientadora: Dr. Gabriela Elisa Kirsch

1. Aterosclerose subclínica. 2. Óxido nítrico. 3. Medicina Alternativa. I. Klafke, Jonatas Zeni. II. Kirsch, Gabriela Elisa. III. Título.

CDU 615.89

Catalogação na fonte: Bibliotecária Eliane C. Reck da Rosa CRB-10/2404

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A Comissão Examinadora, abaixo assinada,

aprova a Dissertação de Mestrado

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elaborada por

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Como requisito parcial para obtenção do grau de

Mestre em Atenção Integral à Saúde

_____

Prof. Dr. Jonatas Zeni Klafke (orientador / UNICRUZ)

COMISSÃO EXAMINADORA

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Cruz Alta, 2016

AGRADECIMENTOS

Primeiramente agradeço a Deus pela imensa bondade de me dar o privilégio de estar neste plano e poder desfrutar das belezas da vida.

Agradeço aos meus pais Simone e Tarcísio, pela oportunidade, por sempre estarem ao meu lado apoiando minhas decisões e me aconselhando, mostrando o melhor caminho a seguir. Vocês são meus heróis!

Agradeço aos meus avós Luci Alves da Silva e Juvino de Almeida, vocês são meus motivos para seguir em frente. Amo vocês.

Agradeço a família que formei morando nesta cidade (Cruz Alta –RS) sendo ela todas as minhas amigas e amigos, em especial Bruna de Bortoli, Ana Paula Martins, Kristine Gonçalves, Sabrina Nascimento e Mariana Parisi, as quais me acompanharam nesta jornada desde o início.

Agradeço, em especial, a Gabriela Elisa Hirsch, minha co-orientadora, colega de pesquisa e principalmente amiga, que esteve comigo em todos os altos e baixos deste curso de pós graduação, me acolhendo e dando força para continuar.

Agradeço ao meu orientador Jonatas Zeni Klafke que está ao meu lado há anos, obrigada pela orientação, paciência, compreensão, disposição, apoio e ajuda disponibilizados em todos os momentos.

Agradeço a todos os membros do Grupo Multidisciplinar em Saúde (GMS) pelas pesquisas que desenvolvemos juntos.

Agradeço ao Programa de Pós Graduação em Atenção Integral à Saúde, as universidades responsáveis pelo programa, UNICRUZ e UNIJUÍ, aos colegas de mestrados e aos professores por todo conhecimento adquirido nas mais diversas áreas.

RESUMO

As doenças cardiovasculares (DCVs) são consideradas a principal causa de mortalidade no mundo, sendo o infarto agudo do miocárdio o mais significante. A aterosclerose subclínica, através da evolução de fatores de risco resulta em doenças cardiovasculares evidentes. O longo perído entre o início da aterosclerose subclínica e manifestação da doença é o principal problema das DCVs Dessa maneira, os biomarcadores são úteis para melhor identificar os indivíduos de risco e diagnosticar as condições da aterosclerose subclínica com mais rapidez e precisão. Uma das propostas de biomarcadores é a albumina modificada por isquemia (IMA), sendo considerada um biomarcador bioquímico promissor para as condições ateroscleróticas. Outro marcador que está ganhando força e está associado a IMA são os produtos de proteína na de oxidação avançada (AOPP), sua medição fornece informações sobre o nível de exposição a alterações potencialmente prejudiciais às proteínas e controle metabólico. E por último mas não menos importante temos óxido nítrico (ON) como marcador precoce, principalmente relacionado à disfunção endotelial. Com isso, também é evidenciado neste trabalho o uso da *Campomanesia xanthocarpa,* uma planta nativa da região sul do Brasil amplamente utilizada como medicina alternativa e complementar, sendo um produto natural utilizado para reduzir a oxidação de proteínas e melhorar a disponibilidade de óxido nítrico e consequentemente a função vascular, reduzindo o risco de desenvolvimento de DCV. Com isso evidencia-se a relação do profissional biomédico no desenvolvimento e estudo científico sobre os biomarcadores de estresse oxidativo e endotelial, permitindo assim a atualização deste profissional e sua relação no desenvolvimento de terapias alternativas relacionadas a aterosclerose subclínica.

Palavras chaves: Aterosclerose subclínica. Proteína de Oxidação Avançada. Albumina Modificada por Isquemia. Óxido Nítrico. Produtos Naturais. Guavirova.

ABSTRACT

Cardiovascular diseases (CVDs) are considered a leading cause of death in the world, with acute myocardial infarction being the most significant. Subclinical atherosclerosis, through the evolution of risk factors results in obvious cardiovascular diseases. The long term between the onset of subclinical atherosclerosis and disease manifestation is the main problem of CVDs. In this way, biomarkers are useful to better identify individuals at risk and diagnosis as conditions of subclinical atherosclerosis more quickly and accurately. One of the proposed biomarkers is an albumin modified by ischemia (IMA), being considered biochemical biomarker promising for the atherosclerotic conditions. Another marker that is gaining strength and is associated with IMA is Advanced Oxidative Protein Products (AOPP), its information on the level of exposure and metabolic potential. And last but not least we have nitric oxide (ON) as an early marker, mainly related to endothelial dysfunction. The use of xanthocarpa, a plant native to the Brazilian region widely used as alternative and complementary medicine, is a natural product used to reduce the oxidation of proteins and to improve the availability of nitric oxide and, consequently, A vascular function, reducing the risk of developing CVD. This shows a relationship of the non-developmental biomedical professional and scientific study on the biomarkers of oxidative and endothelial stress, thus allowing an updating of this professional and their relationship not developing alternative therapies related to subclinical atherosclerosis.

Key words: Atherosclerosis Subclinical. Advanced Oxidation protein. Albumin modified by Ischemia. Nitric oxide. Natural products. Guavirova.

LISTA DE ABREVIATURAS

DCNTs: doenças crônicas não transmissíveis

DCVs: doenças cardiovasculares

LDL: lipoproteína de baixa densidade

AOPP: produto de proteína de oxidação avançada

IMA: albumina modificada por isquemia

ON: óxido nítrico

EROs: espécies reativas de oxigênio

MAC: medicina alternativa e complementar

OMS: organização mundial da saúde

SUMÁRIO

1 INTRODUÇÃO

De acordo com relatórios das Nações Unidas, as doenças crônicas transmissíveis (DCNTs), como as doenças cardiovasculares (DCVs) e o câncer, levaram ao óbito de aproximadamente 38 milhões de pessoas em 2012, representando 68% de todas as mortes e tendendo a aumentar para 52 milhões até 2030. As DCVs foram responsáveis por 17,5 milhões desses óbitos (46,2%) a nível mundial em 2012 e, embora as DCNTs sejam muitas vezes consideradas “doenças das classes mais ricas”, mais de 80% dos óbitos ocorrem em países de renda baixa e média. Ainda, estima-se que em 2030, o número anual global de mortes por DCVs subirá para 23,6 milhões (MENDIS *et al.*, 2011).

Embora as DCNTs sejam responsáveis por altos índices de óbito, quase metade delas (42%) ocorrem de forma prematura e são evitáveis (MENDIS *et al.,* 2011). Da mesma maneira, as DCVs também podem ser evitadas, embora ainda sejam a principal causa de morbidade e mortalidade por DCNTs de acordo com a Organização Mundial da Saúde, e sendo importante salientar que, devido as ações preventivas, os óbitos por doenças relacionadas com o coração estão diminuindo nos países desenvolvidos (BUTLER, 2011; MENDIS *et al.,* 2011; ROGER *et al.,* 2011). Apesar deste recente declínio na mortalidade por DCV em homens e mulheres, a manifestação aguda deste distúrbio pode ocorrer como síndrome coronariana aguda (termo geral utilizado para descrever as condições de isquemia aguda do miocárdio causado pela oclusão de uma artéria coronária), levando a desfechos súbitos e fatais em 30-50% dos sujeitos acometidos (ERBEL *et al.,* 2010; WEBSTER *et al.,* 1990).

Normalmente, a ocorrência de síndrome coronária aguda ocorre devido à formação de um trombo no local de uma placa aterosclerótica de alto risco, que restringe parcialmente ou completamente o fluxo de sangue na artéria coronária afetada, sendo que, aproximadamente 20% do primeiro e dos recorrentes infartos agudos do miocárdio são silenciosos (AMSTERDAM *et al.,* 2014; BOLAND *et al.,* 2002; THOM *et al.,* 2001). O risco de ocorrer um infarto agudo do miocárdio após os 40 anos de idade, período de principal risco para DCV, é de 49% para os homens e de 32% para as mulheres (LLOYD *et al.,* 1999). Ainda, com a utilização do status socioeconômico como medida para o estabelecimento do Índice de Desenvolvimento Humano, pode-se perceber que a prevalência de DCVs está aumentada nos países em desenvolvimento, enquanto há um declínio nos países desenvolvidos (ZHU *et al.,* 2015).

Associado a isso, mais de 300.000 mortes súbitas ocorrem anualmente na população geral dos Estados Unidos, o qual possui aproximadamente 10 vezes mais pacientes definidos como propensos a morte súbita, especialmente devido à baixa fração de ejeção (insuficiência cardíaca sistólica), arritmias ventriculares e infarto agudo do miocárdio (HUIKURI *et al.,* 2001). Este fato tem atraído à atenção de especialistas na área, porque mesmo os cardiologistas de renome acabam por sofrer de morte súbita (SOLER-SOLER, POOLE-WILSON, 2009; DZAU *et al.,* 2009).

Dentre as DCV, destaca-se a aterosclerose que é uma doença inflamatória associada com a ativação das células endoteliais, ao estresse oxidativo e ao acúmulo de leucócitos nas paredes das artérias de grande calibre (IIYAMA *et al.,* 1999; HANSSON *et a.,* 1991). Além disso, há evidências consideráveis de que a aterosclerose pode ter algumas origens no útero

(MATTURI *et al.,* 2004). Nesse contexto, antes da doença ostensiva, a aterosclerose apresenta período subclínico, o qual se caracteriza pela ausência de sintomas clínicos. Os dados substanciais indicam que a aterosclerose subclínica é uma doença que começa com a evolução de fatores de risco que contribuem para o desenvolvimento das DCVs (RAITAKARI *et al.,* 2003; PSATY *et al.,* 1999; BERENSON *et al.,* 1998; KULLER *et al.,* 1995). A hipercolesterolemia, um dos principais fatores de risco para aterosclerose, pode refletir eventos que ocorrem nas células endoteliais de todo os sistema vascular, podendo inclusive representar uma resposta que inicia ou perpetua eventos inflamatórios observados em segmentos propensos à lesão no sistema arterial. A hipercolesterolemia caracteriza-se pelo nível de colesterol total acima de 240mg/dl e a lipoproteína de baixa densidade (LDL) acima de 160 mg/dl (DAVIDSON *et al.,* 2003).

Nesse sentido, tem sido proposto que um baixo grau de inflamação rapidamente induzido pela hipercolesterolemia pode promover o acúmulo de mediadores inflamatórios na circulação sistêmica, produzido pelas células circulantes que interagem com as células endoteliais, as quais revestem as grandes artérias, e, finalmente, contribuindo com a patogênese da aterosclerose subclínica (STOKES *et al.,* 2002).

No entanto, além da inflamação, o estresse oxidativo também contribui para a disfunção vascular no processo aterogênico, sendo que várias evidências indicam que a oxidação proteica e seu acúmulo subsequente nas células representa uma indicação precoce de lesão tissular mediada pelo radical oxigênio, ocorrendo durante períodos de estresse oxidativo e na aterosclerose (NEDELJKOVIC *et al.,* 2003; STADMAN, LEVINE, 2003).

O principal problema relacionado às DCVs ocorre devido ao largo tempo entre o início da aterosclerose subclínica e a manifestação da doença muitas décadas depois (STARY, 1990; FUSTER *et al.,* 1989). A identificação dos indivíduos em risco para tais eventos é claramente importante, pois pode levar a implementação e cumprimento de medidas preventivas eficazes para reduzir o risco da doença (FALK *et al.,* 1995).

Dessa maneira, os biomarcadores podem ser úteis na identificação de indivíduos com risco de DCV, e no diagnóstico das condições da doença com maior rapidez e precisão, sendo que os pacientes podem, então, ser aconselhados a mudar seu estilo de vida, a fim de evitar uma maior progressão da doença, especialmente quando os biomarcadores estiverem alterados. No entanto, poucos biomarcadores e métodos de diagnóstico adequados estão disponíveis para identificar esta patologia progressiva durante seus estágios iniciais, e o conjunto existente de agentes farmacêuticos não permite ao médico realizar uma intervenção preventiva no indivíduo que é considerado saudável no presente momento, a fim de prevenir a progressão da doença. Para tanto, novos biomarcadores da aterosclerose subclínica podem indicar uma variedade de características da doença, incluindo os aspectos oxidativos e da função vascular (FOX, GROWDON, 2004).

Em termos de biomarcadores de estresse oxidativo, dentre muitos que vem sendo estudados, existem aqueles que ainda não são aplicados na clínica, sendo apenas aplicados em laboratórios de pesquisa, os quais vem demonstrando fortes evidências para o aspecto subclínico das DCVs. As proteínas estão continuamente sob condições oxidantes, em tecidos humanos e fluidos corporais, o que resulta na formação de baixos níveis de produtos

oxidados e com o desbalanço característico do estresse oxidativo, ocorre maior formação de proteínas oxidadas e com isso é possível a detecção do biomarcador de estresse oxidativo conhecido como produto de proteína de oxidação avançada (AOPP) é utilizado como uma medida de níveis elevados de proteínas oxidadas, particularmente a albumina (THORNALLEY, RABBANI, 2014; WITKO-SARSAT *et al.,* 1996). O AOPP tem sido altamente correlacionado com a espessura da íntima-média da carótida e pode até ser relacionada a eventos cardiovasculares ateroscleróticos (DESCAMPS-LATSCHA *et al.,* 2005). Além disso, tem sido sugerido que o AOPP representa uma nova classe de mediadores pró-inflamatórios e atua como fonte de mais estresse oxidativo e ativação de monócitos (KASISKE, 1998).

O excesso de produção de radicais livres pode produzir modificações químicas da albumina no soro humano, resultando em aumento do biomarcador conhecido como albumina modificada por isquemia (IMA) (ZHONG *et al.,* 2012). Atualmente a IMA é considerada como um biomarcador de estresse oxidativo, que está relacionado com a isquemia de reperfusão em uma variedade de condições clínicas associadas ao estresse oxidativo, tais como a isquemia do miocárdio, síndrome metabólica e hipercolesterolemia (GOTTLIEB *et al.,* 2010; DUARTE *et al.,* 2009; BAR-OR *et al.,* 2000).

Uma característica central dessas condições oxidativas é a função endotelial prejudicada, uma vez que os fatores de risco cardiovasculares, tais como hipercolesterolemia, hipertensão e diabetes mellitus aumentam a geração de espécies reativas de oxigênio (EROs), os quais, por sua vez, reduzem a biodisponibilidade do óxido nítrico (ON) derivado do endotélio, que desempenha um papel protetor na vasculatura, sendo conhecido pelo seu

importante papel vasodilatador (ARMSTRONG *et al.,* 2006).

O ON é também envolvido na inibição da adesão de plaquetas e agregação, podendo inclusive reduzir a aderência de leucócitos ao endotélio vascular e suprimir a proliferação de células de músculo liso vascular através da inibição de fatores de crescimento. O reconhecimento das enzimas de síntese do ON é importante para a compreensão dos mecanismos fisiopatológicos associados ao ON em doenças cardiovasculares, sendo que estas enzimas podem ser alvos para novas intervenções terapêuticas (COOKE e DAZU, 1997).

Dessa forma, a evolução destes biomarcadores oxidativos (AOPP e IMA) e endotelial (ON) é importante na detecção precoce do quadro de aterosclerose subclínica, possibilitando também o monitoramento de diferentes tratamentos, inclusive aqueles capazes de modificar os fatores de risco cardiovasculares.

Cabe salientar que na maioria das vezes o indivíduo está acometido por diferentes fatores de risco cardiovasculares para além da hipercolesterolemia. Além disso, a permanência destes fatores e a baixa adesão das mudanças de estilo de vida é frequentemente responsável pelo considerável aumento das manifestações cardiovasculares (BLASCHKE *et al.,* 2012). Sendo assim, melhorar a adesão do paciente a intervenções eficazes como a prática de atividade física e reeducação dietética, por meio de alterações comportamentais, é um grande desafio de saúde global, envolvendo um investimento intensivo de tempo e recursos por profissionais de saúde (GRANGER, BOSWORTH, 2011).

Além disso, os tratamentos farmacológicos convencionais geralmente

são de alto custo, como por exemplo o uso de estatinas, os quais acabam progressivamente tornando-se menos eficazes ao longo do tempo, além de levar o indivíduo ao risco de desenvolver miopatias fazendo com que os pacientes busquem pelo uso da medicina alternativa e complementar (MAC) (GRANGER, BOSWORTH, 2011).

A MAC é definida como um grupo de sistemas, de práticas e de produtos para saúde que não são considerados parte da medicina convencional, como por exemplo: sistemas médicos alternativos (homeopatia); intervenções mente-corpo (meditações, orações); terapias biológicas (baseados em produtos naturais não reconhecidos cientificamente); métodos de manipulação corporal e baseados no corpo (massagens, exercícios); e terapias energéticas (reiki). Quando essas práticas são utilizadas juntamente com as práticas médicas convencionais, são denominadas complementares; quando são usadas no lugar de uma prática médica convencional, são denominadas alternativas; sendo que quando são usadas conjuntamente baseadas em avaliações científicas de segurança e eficácia de boa qualidade, são chamadas integrativas (BARNES *et al.,* 2008).

A MAC está sob a determinação da OMS, a qual recomenda que os países desenvolvam políticas nacionais que visam a integração e a inclusão da MAC nos sistemas oficiais de saúde, com foco na Atenção Primária à Saúde (OMS, 2002a; OMS, 2002b). Assim, muitos esforços estão sendo realizados no campo dos produtos naturais como MAC no controle de DCVs, contribuindo inclusive na melhoria dos biomarcadores relacionados a aterosclerose subclínica.

Nesse sentido, considerando o exposto e o crescente aumento do uso

de plantas na MAC em todo mundo, evidencia-se a existência de poucos estudos clínicos de plantas nativas brasileiras que realmente demonstram eficácia em atuar na melhoria de biomarcadores relacionados a aterosclerose subclínica. Nesse contexto destaca-se a planta *Campomanesia xanthocarpa* Berg. (Myrtaceae), a qual vem sendo alvo de estudo em nosso grupo de pesquisa por apresentar efeitos cardiovasculares de melhora em parâmetros envolvendo a aterosclerose.. A *C. xanthocarpa* pertence à família *Myrtaceae*, a qual compreende cerca de 3500 espécies, subordinadas a mais ou menos 100 gêneros e apresenta dois centros principais de desenvolvimento: a América tropical e a Austrália (BARROSO, 1991). Dentre seus nomes populares destacam-se “guabirobeira”, “guabiroba”, “guabirova”, “guabirobeira-do-mato”, “guaribagabirobeira” (LORENZI, 1992).

A planta é decídua (não perde as folhas facilmente), mesófita até heliófita (desenvolve-se na presença de luz), e seletiva higrófita, sendo abundante nas partes úmidas das matas de altitude. Ocorre em Minas Gerais, São Paulo, Mato Grosso do Sul até o Rio Grande do Sul, em quase todas as formas florestais. Floresce abundantemente durante os meses de setembro-novembro e os frutos amadurecem em novembro-dezembro. Os frutos da guabirobeira, de acordo com os estudiosos, além de saborosos, são também ricos em vitamina C. O fruto pode ser consumido *in natura* ou na forma de sucos, doces, geléias e ainda serve como matéria-prima para licores, picolés e sorvetes (BIAVATTI, 2004; LORENZI, 1992).

Em um estudo piloto pioneiro, o pó das folhas de *C. xanthocarpa* produziu efeito semelhante ao mecanismo das estatinas, uma vez que reduz colesterol LDL e inibindo a atividade da enzima HMG-coa redutase (KLAFKE *et*

al., 2010), ratificando a crendice popular do efeito hipolipemiante desta planta em indivíduos hipercolesterolêmicos, abrindo, assim, possibilidades para a ampliação de maiores investigações sobre essa planta. Dentro dessa premissa, mais recentemente, fora confirmado em recente estudo clínico o efeito hipocolesterolêmico dessa planta, através do estudo de Viecili e colaboradores (2014), o qual envolveu um grande número de indivíduos hipercolesterolêmicos, demonstrando inclusive que a planta possui efeito antioxidante, anti-inflamatório e de melhora da biodisponibilidade dos níveis de óxido nítrico nestes indivíduos. Cabe salientar que em ambos estudos, o tratamento com a *C. xanthocarpa* ocorreu por meio da administração do pó encapsulado das folhas da planta durante 90 dias (VIECILI *et al.,* 2014; KLAFKE *et al.,* 2010).

Além desses estudos, a planta atuou demonstrando efeito antiagregante plaquetário, antitrombótico e fibrinolítico em um estudo *in vitro* e em modelo animal (KLAFKE *et al.,* 2012) e também demonstrou efeito anti-inflamatório e antioxidantes em camundongos knockout para o receptor LDL quando comparado com o ácido acetilsalicílico (KLAFKE *et al.,* 2016). Em análise cromatográfica, por HPLC-DAD, usando o extrato da *C. xanthocarpa*, foi encontrado a presença de flavonoides e ácidos fenólicos. Os compostos mais abundantes presentes no extrato foram ácidos fenólicos: ácido gálico e ácido clorogênico; e além destes, os flavonoides encontrados foram rutina, quercetina e kampeferol (KLAFKE *et al.,* 2016). Um outro estudo demonstrou que o flavonoide e o ácido fenólico, mais abundantes, encontrados nas folhas da planta são, respectivamente, quercetina e ácido gálico (KARAOKA e CARDOSO, 2013). Estes compostos são responsáveis por diminuir a produção

de radicais livres ou neutralizá-los, possuindo propriedades antioxidante reconhecidas, os quais podem contribuir para bons resultados descritos para espécie.

Nesse sentido, é notório, nos últimos anos, a presença do profissional Biomédico juntamente com a equipe multiprofissional no desenvolvimento de pesquisas relacionadas a novos biomarcadores para DCNTs, bem como também no processo de prevenção e tratamento das mesmas, especialmente das DCVs. Dessa maneira, ressalta-se a importância do Biomédico no estudo de biomarcadores para diagnóstico da aterosclerose subclínica, buscando inclusive contribuir multiprofissionalmente com a descoberta de novos tratamentos alternativos e complementares para essa patologia subclínica.

Este trabalho justifica-se pelo fato de revisar importantes biomarcadores oxidativos e endotelial de pesquisa relacionados à aterosclerose subclínica atualmente, contribuindo para fortalecer evidências sobre biomarcadores específicos que possam ser utilizados futuramente para prevenção e diagnóstico, bem como sugerir importante tratamento alternativo e complementar para melhorar as condições oxidativas e endotelial envolvidos na aterosclerose.

Dessa forma, baseado na hipótese científica de que existem biomarcadores utilizados laboratorialmente em pesquisa relacionados à aterosclerose subclínica, bem como tratamentos alternativos e complementares para essa condição, o objetivo desse estudo foi revisar e compilar informações acerca de biomarcadores oxidativos e endotelial relacionados a pesquisa em aterosclerose subclínica, avaliando-os como possíveis biomarcadores para o

diagnóstico subclínico e para o monitoramento de terapia alternativa e complementar, principalmente através da planta *Campomanesia xanthocarpa.*

2 OBJETIVOS

2.1 Objetivo Geral

Revisar e compilar informações acerca de biomarcadores oxidativos e endotelial relacionados a pesquisa em aterosclerose subclínica avaliando-os como possíveis biomarcadores para o diagnóstico subclínico e para o monitoramento de terapia alternativa e complementar, principalmente através da planta *Campomanesia xanthocarpa.*

2.2 Objetivos Específicos

- Demonstrar a importância e novos biomarcadores na detecção precoce da aterosclerose subclínica;

- Descrever sob o ponto de vista contextual científico os biomarcadores oxidativos AOPP e IMA bem como o biomarcador endotelial ON, relacionando-os a aterosclerose subclínica.

- Verificar a aplicabilidade dos biomarcadores de aterosclerose subclínica para serem usados na detecção precoce deste patologia,

- Evidenciar a utilização dos tratamentos alternativos e complementares na redução da mortalidade por DCVs;

- Compilar e discutir o envolvimentos da *C. xanthocarpa* no tratamento alternativo e complementar da aterosclerose subclínica;

- Contribuir com a produção científica na área de etnofarmacologia, esclarecendo atividades químicas e biológicas da *C. xanthocarpa* do aspecto vascular;

- Permitir a atualização do profissional biomédico acerca dos biomarcadores e produtos naturais como tratamento alternativo e complementar para aterosclerose subclínica;

- Debater os temas em equipe interprofissional para criar habilidades e competências interdisciplinares para a formação do discente com foco na Atenção Integral à Saúde;

3 METODOLOGIA

3.1 Revisão Narrativa

A revisão narrativa é um tipo de revisão bibliográfica a qual conduz à análise crítica, meticulosa e ampla das publicações correntes em uma determinada área do conhecimento (TENTINI e PAIM, 1999). A revisão narrativa permite a construção de uma publicação ampla e é apropriada para descrever e discutir o desenvolvimento ou o "estado da arte" de um determinado assunto, sob ponto de vista teórico ou contextual. Este tipo de revisão constitui-se, basicamente, de análise de literatura publicada em livros e artigos de revistas impressas e/ou eletrônicas que são submetidos à interpretação e análise crítica pessoal do autor (ROTHER, 2007).

Para Fogliatto (2007), a revisão bibliográfica é aquela que reúne ideias oriundas de diferentes fontes, visando construir uma nova teoria ou uma nova forma de apresentação para um assunto já conhecido. A pesquisa bibliográfica procura explicar e discutir um tema com base em referências teóricas publicadas em livros, revistas, periódicos e outros. Busca também, conhecer e analisar conteúdos científicos sobre determinado tema. Deste modo, a pesquisa bibliográfica não é apenas uma mera repetição do que já foi dito ou escrito sobre determinado assunto, ela proporciona o exame de um tema sob novo enfoque ou abordagem, chegando a conclusões inovadoras (MARTINS e PINTO, 2001).

Desse modo, optou-se pela revisão bibliográfica do tipo narrativa, a qual tem papel fundamental para a educação continuada, uma vez que permite ao leitor adquirir e atualizar o conhecimento sobre uma temática específica em curto intervalo de tempo. Cabe salientar que a revisão narrativa não possui

metodologia que permitam a reprodução dos dados e nem fornece respostas quantitativas para questões específicas, sendo, deste modo, considerados artigos de revisão narrativa e qualitativa (TENTINI e PAIM, 1999).

Na elaboração deste trabalho foi realizada uma revisão narrativa de literatura internacional sobre o tema proposto, a qual foi constituída pelos seguintes subtemas:

- Medicina Alternativa e Complementar no tratamento da aterosclerose
- Biomarcadores oxidativos e inflamatórios como preditores de doença e da função vascular
- Produto de proteína de oxidação avançada
- Albumina modificada por isquemia
- Óxido nítrico
- Fundamentos para o uso de um produto natural como medicina alternativa e complementar
- *Campomanesia xanthocarpa* como MAC no tratamento da aterosclerose

3.2 Levantamento dos dados

Bases para consulta: PUBMED (*National Center for Biotechnology Information*, U.S. *National Library of Medicine*), LILACS (Literatura Latino Americana e do Caribe em Ciências Sociais e da Saúde), Scielo (*Scientific Eletronic Library OnLine*) serviram como instrumento para coleta de dados.

3.3 Amostra

A amostra do estudo foi composta por toda a literatura relacionada ao tema de estudo, indexada nos bancos de dados PUBMED, LILACS, Scielo, obtida a partir da pesquisa realizada. A seleção foi realizada a partir de leitura criteriosa dos artigos e livros encontrados nas bases de dados.

Foram incluídas apenas as publicações relacionadas aos descritores pesquisados envolvidos com o tema central do estudo, sendo que, preferencialmente, serão utilizadas literaturas atuais, não se descartando, no entanto, a possibilidade de utilização da literatura clássica no idioma inglês.

3.4 Equipe Multiprofissional

Foi formada uma equipe multiprofissional que realizou a leitura e análise crítica dos artigos e livros oriundos da busca nos bancos de dados elencados. A equipe contou com quatro biomédicos da Universidade de Cruz Alta –RS, sendo dois professores e dois alunos do Mestrado em Atenção Integral à Saúde, duas farmacêuticas, sendo uma Pós Doutoranda do Programa de Pós Graduação em Atenção Integral à Saúde e outra professora da Universidade Federal de Santa Maria – RS, e por um médico cardiologista do Instituto de Cardiologia de Cruz Alta –RS.

A formação desta equipe teve por objetivo dinamizar o conhecimento e a síntese dos estudos elencados, de forma a garantir que se obtivesse um panorama amplo e dinâmico do assunto sob diferentes pontos de vista.

3.5 Centros de Pesquisa envolvidos

3.5.1 Grupo Multidisciplinar de Saúde – Universidade de Cruz Alta

O grupo de pesquisa está cadastrado no Diretório dos Grupos de Pesquisa do Conselho Nacional de Desenvolvimento Científico e tecnológico e é composto por acadêmicos, técnicos e docentes vinculados à Universidade de Cruz Alta-RS. Os resultados da implantação do Grupo Multidisciplinar de Saúde da UNICRUZ podem ser visualizados pela produção científica dos pesquisadores com publicações nacionais e internacionais e pela quantidade de trabalhos científicos que estão sendo apresentados em eventos internacionais, nacionais e regionais.

3.5.2 Grupo Interdisciplinar de Saúde - Centro de Ensino e Pesquisa do Instituto de Cardiologia de Cruz Alta (CEP-ICCA)

O grupo de pesquisa do Centro de Ensino e Pesquisa do Instituto de Cardiologia de Cruz Alta cadastrado no Diretório dos Grupos de Pesquisa do Conselho Nacional de Desenvolvimento Científico e tecnológico e está organizado com pesquisadores, estudantes e profissionais de alto nível acadêmico e intelectual de instituições distintas, em permanente interação, com reconhecida competência e tradição em suas áreas de atuação técnico-científica, que funcionam como fonte geradora e transformadora de conhecimento científico-tecnológico para aplicação em projetos de relevância à promoção da saúde. O grupo tem por objetivos atuar em pesquisa, extensão e pós-graduação na área da saúde.

4 ARTIGO CIENTÍFICO

Os resultados inseridos nesta dissertação apresentam-se sob a forma de artigo de revisão científico, o qual se encontra neste capítulo apresentado. Tratou-se de um artigo de revisão narrativa, o qual se encontra publicado no periódico internacional Current Pharmaceutical Design ISSN 1381 6128 (fator de impacto 3.052).

5 CONSIDERAÇÕES FINAIS

A busca por novos biomarcadores para aterosclerose subclínica tem demonstrado avanço rumo a novas descobertas úteis para o tratamento de processos ateroscleróticos subclínicos. Dessa forma os biomarcad oxidativos (AOPP e IMA) e endotelial (ON) estão relacionados a aterosclerose subclínica, no entanto para uso rotineiro na clínica estudos adicionais são necessários para padronizar a sua precisão (sensibilidade, especificidade e valores preditivos). A avaliação dos níveis de AOPP, IMA e ON pode ajudar a melhorar a detecção do risco cardiovascular subclínico, no entanto ainda precisam se tornar disponíveis a um baixo custo, estando acessíveis não apenas em laboratórios de pesquisa, mas em laboratórios de diagnóstico para ser utilizado em todos os níveis de saúde.

A MAC está avançando por meio de estudos que envolvem plantas e produtos naturais, sendo que alguns destes produtos podem agir na redução da mortalidade cardiovascular através de diferentes mecanismos intimamente relacionados a aterosclerose subclínica. Dentro desses estudos a *C. xanthocarpa* demonstra sua ação em diferentes mecanismos da aterosclerose subclínica, reduzindo a oxidação de proteínas e melhorando a biodisponibilidade do óxido nítrico e, consequentemente, a função vascular. Embora a *C. xanthocarpa* apresente fortes evidências científicas acerca da aterosclerose, podendo ser utilizada na MAC, sua utilização ainda depende da realização de inúmeros estudos exigidos pelas agências reguladoras para que se torne um produto comercial fitoterápico.

Esta revisão buscou em diversos artigos informações sobre o tema proposto, as quais foram compiladas, contribuindo assim para a produção

científica na área de etnofarmacologia, através de uma revisão narrativa.

Pode ser evidenciado a relação do profissional biomédico no desenvolvimento e estudo científico sobre os biomarcadores de estresse oxidativo e endotelial, permitindo assim a atualização deste profissional e sua relação no desenvolvimento de terapias alternativas relacionadas a aterosclerose subclínica, e além disso, para a produção deste trabalho, houve a participação de diversos profissionais da área da saúde, os quais debateram sobre o assunto interdisciplinarmente, demonstrando assim que a temática é fundamental para a Atenção Integral à Saúde.

6 PERSPECTIVAS FUTURAS

Através da pesquisa bibliográfica realizada nesta dissertação, foi possível verificar que os biomarcadores oxidativos AOPP, IMA e endotelial ON são importantes biomarcadores disponíveis na pesquisa relacionada à aterosclerose subclínica atualmente, existindo fortes evidências de que refletem aspectos relacionados aos eventos oxidativos e endotelial crucias para a aterosclerose subclínica.

Para tanto, pretende-se avaliar em modelo experimental se esta hipótese confirma-se por meio de estudos que já se encontram em fase de desenvolvimento pelo nosso grupo de pesquisa. Um desses estudos é "AVALIAÇÃO DA *Campomanesia xanthocarpa* NO ESTRESSE OXIDATIVO E BIODISPONIBILIDADE DE ÓXIDO NÍTRICO EM RATOS HIPERCOLESTEROLÊMICOS", o qual pretende avaliar os efeitos do tratamento com a planta *Campomanesia xanthocarpa* sobre os níveis de AOPP, IMA e ON em ratos que apresentam hipercolesterolemia induzida por dieta. Neste trabalho, espera-se confirmar que a hipercolesterolemia, um importante fator de risco que contribui para aterosclerose subclínica, realmente influencia no aumento dos níveis dos biomarcadores AOPP e IMA e na redução da biodisponibilidade de ON, e que o tratamento com produtos naturais como a *C. xanthocarpa,* são capazes de reverter esse quadro, prevenindo precocemente o desenvolvimento da aterosclerose.

Além disso, pretende-se verificar, em estudo clínico piloto, se a planta *C. xanthocarpa* seria capaz de atuar sobre os biomarcadores AOPP, IMA e ON, além de outros marcadores de estresse oxidativo, em indivíduos sem a presença de fatores de risco para DCVs, ou seja, indivíduos saudáveis.

No caso dos resultados obtidos nestes estudos serem promissores e confirmarem a hipótese apresentada nesta revisão, nós ainda pretendemos conduzir outros trabalhos futuros que busquem estabelecer o uso dos biomarcadores da aterosclerose subclínica na prática clínica rotineira, visando contribuir para a redução da morbimortalidade causada pelas DCVs, através da detecção precoce e prevenção do desenvolvimento desta patologia, sempre em busca da qualidade científica e profissional em atenção integral à saúde.

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Current Pharmaceutical Design, 2016, *22*, 000-000

Biomarkers of Subclinical Atherosclerosis and Natural Products as Complementary Alternative Medicine

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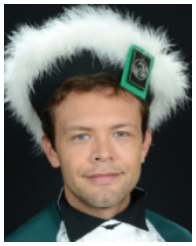

Jonatas Zeni Klafke

Abstract: Cardiovascular diseases (CVD) are considered the leading cause of morbidity and mortality from chronic diseases in the world. In addition, about 20% of first and recurrent acute myocardial infarctions (MI) are silent. In this context, subclinical atherosclerosis culminates in evident CVD, through the evolution of early risk factors such as hypercholesterolemia, hypertriglyceridemia and others. The main problem in CVD is related to the long-time between the start of the subclinical atherosclerosis and the manifestation of the disease. The identification of subjects at risk of such events is obviously substantial, since identification leads to implementation and compliance with effective preventive measures that reduce such risk. In this sense, this review demonstrates biomarkers as an alternative to early detection of subclinical atherosclerosis. One of the proposed biomarkers is the Ischemia-modified albumin (IMA), being considered a promising biochemical biomarker for atherosclerotic conditions. Another marker that is gaining strength and is associated with the IMA are the advanced oxidation protein products (AOPP), its measurement provides information on the level of exposure to potentially harmful changes to proteins and metabolic control. And last but not least we have nitric oxide as an early marker mainly related to endothelial dysfunction. In this review also is evidenced the use of the *Campomanesia xanthocarpa*, a plant native to southern region from Brazil extensively used as complementary and alternative medicine, and natural products to reduce protein oxidation and improve the availability of nitric oxide and consequently vascular function, reducing the risk for development of CVD.

Keywords: Advanced oxidation protein products, *Campomanesia xanthocarpa,* Cardiovascular diseases, Ischemia-modified albumin, Medicinal plant, Nitric oxide.

INTRODUCTION

According to United Nations reports, chronic diseases such as cardiovascular disease (CVD) and cancer killed 36 million people in 2008, accounting for 63% of all deaths. CVDs account for > 17 million deaths globally 2008 (30% of all deaths). Although chronic diseases are often considered to be diseases of affluence, more than 80% of chronic disease deaths occurred in low- and middle-income countries. By 2030, the global annual toll of CVDs will rise to 23.6 million [1, 2].

CVDs are the main cause of morbidity and mortality by chronic disease according to a World Health Organization report [1, 2], however deaths from heart-related diseases are decreasing in developed countries due to preventive actions [3]. Despite a recent decline in CVD mortality in men and women, acute manifestation can occur as acute coronary syndrome, leading to sudden and fatal outcomes in 30-50% of subjects [4, 5]. Acute coronary syndrome is an umbrella term used to describe conditions of acute myocardial ischemia caused by occlusion of a coronary artery. This typically occurs because of the formation of a thrombus at the site of a high-risk atherosclerotic plaque, which partially or completely restricts blood flow through the affected coronary artery [6]. In addition, approximately 20% of first and recurrent acute myocardial infarctions are silent [7, 8]. The lifetime risk of acute myocardial infarct, the largest contributor to CVD, after the age of 40 years is 49% for men and 32% for women [9]. Moreover, with the development of socioeconomic status as measured by the Human Development Index, the prevalence of CVD is increasing in developing countries, but declining in developed countries [10].

Additionally, more than 300,000 sudden cardiac deaths occur annually in the general US population, which is approximately 10 times more in patients who are defined as prone to sudden death due to low ejection fraction, ventricular arrhythmias, and acute myocardial infarction [11]. This has attracted the attention of experts in the field, because even the renowned cardiologists end up suffering from sudden cardiac deaths [12, 13].

Subclinical disease culminates in overt CVD [14, 15], and substantial data indicate that subclinical atherosclerosis is a life course disease that begins with the evolution of risk factors that in turn contribute to CVD development [16, 17]. Hypercholesterolemia, one of the main risk factors for atherosclerosis, may reflect events that occur in endothelial cells throughout the vascular tree or may represent a response that initiates or perpetuates inflammatory events observed in lesion-prone segments of the arterial tree. It has been proposed that the low-grade inflammation rapidly induced by hypercholesterolemia may promote accumulation of inflammatory mediators in systemic circulation, prime circulating cells for interactions with endothelial cells lining large arteries, and ultimately contribute to the pathogenesis of subclinical atherosclerosis [18]. The main problem in CVD is related to the long delay between the start of subclinical atherosclerosis and manifestation of the disease many decades later [19, 20]. The identification of subjects at risk for such events is clearly important, as identification leads to im-

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1381-6128/16 $58.00+.00

plementation and compliance with effective preventive measures to reduce disease risk [21]. Biomarkers can be used to better identify high-risk individuals and diagnose disease conditions promptly and accurately. Patients are advised to change their lifestyles in order to prevent further progression of the disease. However, few adequate biomarkers and diagnostic methods are available to identify the progressive pathology during very early stages, and the existing set of pharmaceutical agents does not allow for a physician to perform preventive intervention in an individual who is considered healthy at the present time. New biomarkers of subclinical atherosclerosis can indicate a variety of health or disease characteristics, including the level or type of exposure to an environmental factor, genetic susceptibility, genetic responses to exposures, markers of subclinical or clinical disease, or indicators of response to therapy [22].

Among all modifiable circumstances, poor adherence is frequently responsible for considerable treatment failure and is grouped under the term residual risk. Adherence remains extremely low and is frequently underestimated despite the availability of fairly sophisticated methods of documentation [23]. Improving patient adherence shares some characteristics with improvements in physical activity and diet in that they are behavioral modifications that are major global health challenges and are partially effective interventions involving an intensive investment of time and resources by health professionals; these methods are generally cost-prohibitive and become progressively less effective over time [24].

Atherosclerosis is an inflammatory disease associated with endothelial cell activation, oxidative stress, and the accumulation of leukocytes in the walls of large arteries [25-27]. Additionally, there is considerable evidence that atherosclerosis may have some origins *in utero* [28].

In addition to inflammation, oxidative stress is also known to contribute to vascular dysfunction in the atherogenic process [29]. Several lines of evidence indicate that protein oxidation and the subsequent accumulation of oxidized proteins in cells, which may represents an early indication of oxygen-radical-mediated tissue damage, occurs during periods of oxidative stress and atherosclerosis [30]. The oxidative stress biomarker advanced oxidation protein products (AOPP) are used as a measure of high levels of oxidized proteins, particularly albumin [31]. AOPP have been highly correlated with carotid intima-media thickness and may even be related to atherosclerotic cardiovascular events [32]. Moreover, it has been suggested that AOPP represent a novel class of proinflammatory mediators and act as sources of further oxidative stress and monocyte activation [33].

Furthermore, overproduction of free radicals may produce chemical modifications of human serum albumin, resulting in increased ischemia-modified albumin (IMA) [34]. Currently, IMA is regarded as a biomarker of oxidative stress that is related to ischemia-reperfusion in a variety of clinical conditions associated with oxidative stress, such as myocardial ischemia [35], metabolic syndrome [36], and hypercholesterolemia [37]. A central feature of these conditions is impaired endothelial function. Cardiovascular risk factors such as hypercholesterolemia, hypertension, and diabetes mellitus enhance reactive oxygen species (ROS) generation resulting in oxidative stress. Oxidative stress leads to impaired bioactivity of endothelium-derived nitric oxide (NO), which plays a protective role in the vasculature [38]. This impairment leads to oxidative modification of lipoproteins and phospholipids, contributing to atherogenesis [39]. The evolution of these biomarkers (AOPP, IMA, and NO) is important in the early detection of the atherosclerosis framework, thus supporting the inclusion of an adjuvant treatment for subclinical atherosclerosis, particularly medicinal plants, aimed at improving patient adherence intervention and quality of life, as well as reducing the morbidity and mortality caused by the disease and its complications.

COMPLEMENTARY AND ALTERNATIVE MEDICINE IN ATHEROSCLEROSIS TREATMENT

Patients with classic risk cardiovascular factors are at an increased risk of developing premature cardiovascular disease. Despite significant advances in the understanding of the effects of atherosclerosis on the vasculature, clear guidelines for the management of complementary and alternative approaches of cardiovascular risk factors in patients with subclinical atherosclerosis are limited. Thus, rigorous studies assessing the individual contributions of complementary and alternative approaches used in subclinical atherosclerosis are necessary. Furthermore, effective screening methods are needed to identify patients with subclinical atherosclerosis who are at the highest risk for atherosclerotic complications as well as those who may benefit from early intervention. Advances in the understanding of vascular damage indicate that disease-specific preventive strategies can be developed to ameliorate or abrogate premature cardiovascular disease in these patients.

Many therapeutic agents are available for the management of hypercholesterolemic individuals. Some studies have demonstrated that the use of lipid-lowering drugs can reduce the number of cardiovascular events and mortality from coronary disease [40, 41]. Moreover, a low-cholesterol diet and regular physical exercise should be proposed as adjuvants in the treatment of these individuals [42]. However, because of resistance to dietary restrictions and financial limitations to the use of lipid-lowering drugs, many individuals have turned to alternative treatments to control cholesterol levels. Many of these alternative treatments have been used empirically and have not been thoroughly analyzed to determine their effectiveness and safety [43].

Complementary and alternative medicine (CAM) refers to a broad range of healing modalities external to the biomedical models of health care with an array of non-prescription products and healthcare services not linked to the medical profession or the medical curriculum [44-46]. According to the National Center for Complementary and Integrative Health [47], "if a non-mainstream practice is used together with conventional medicine, it is considered complementary; if a non-mainstream practice is used in place of conventional medicine, it is considered alternative."

Although no evidence supports its effectiveness, CAM is widely available internationally and has become popular in Australia, the US, and Brazil, as well as in many other countries in recent years [48-50]. CAM application has become a topic of significant international public health investigation [51, 52] and a significant health services issue. CAM includes a large and diverse group of orally or topically administered substances such as herbal medicines, botanicals, and probiotics, referred to in this review as CAM natural products. These products are widely marketed, readily available to consumers, and often sold as dietary supplements. According to the 2012 National Health Interview Survey [53], which included a comprehensive survey to examine the use of complementary health approaches by Americans, 17.7% of American adults had used a dietary supplement other than vitamins and minerals in the past year.

In Brazil, the edible plant *Campomanesia xanthocarpa* Berg. (Myrtaceae), popularly known as "guavirova", is found in the southern regions and also in other countries as Argentina, Paraguay, and Uruguay [54] and has been the main target of study of our research group (the Multidisciplinary Group Health). Studies have shown that *C. xanthocarpa* possesses a wide spectrum of physiological effects; the leaves of this plant are used as infusions in folk medicines to treat inflammatory diseases and hypercholesterolemia [55]. Moreover, *C. xanthocarpa* is used for weight loss and control of a number of conditions associated with obesity [43]. Recent studies have shown that the mechanism of the action of *C. xanthocarpa* is similar to that of oral hypolipemiants [56, 57]. Moreover, *C. xanthocarpa* showed antiplatelet, antithrombotic, and fibrinolytic ac-

tivities in mice [58]. Therefore, the means of such therapy based on the use of non-pharmaceutics to subclinical atherosclerosis, based on complementary and alternative approaches, should be examined.

Thereby, the modern approaches for examining novel diagnostic methods and evolving biomarkers will be discussed in this review aimed at evaluating subclinical atherosclerosis in humans and identifying molecular and pathway targets for further development of therapy with natural products.

INFLAMMATORY AND OXIDATIVE BIOMARKERS AS PREDICTORS OF VASCULAR FUNCTION AND DISEASE

One of the major challenges in managing cardiovascular disease is the early diagnosis of subjects with subclinical atherosclerosis in order to improve treatment and reduced the risk to a symptomatic cardiovascular disease. Thus, early alternative complementary and alternative treatments may reduce mortality and morbidity associated with cardiovascular events. Despite the need for screening of atherosclerosis in the general population, few tools are available for screening of subclinical atherosclerosis in asymptomatic subjects lacking risk factors [60]. For example, a recent case-control study showed that only 13% of women and 50% of men who developed acute myocardial infarction were classified as high-risk patients based on their Framingham scores [60]. In this view, progress in reducing mortality related to cardiovascular diseases may be achieved by checking for relevant biomarkers for routine screening in the general population, simultaneously with developing alternative and complementary pharmacological strategies that are able to modify disease progression.

Previous studies have identified specific biomarkers that are positively correlated with atherosclerosis progression [60-64]. Thus, inclusion of a highly specific and sensitive biomarker in routine clinical examinations of individuals is an important factor for identifying and managing patients with early cardiovascular disease risk [59]. Currently, a set of associated factors are widely used in clinical practice as predictors of cardiovascular disease. These factors include biochemical and inflammatory parameters such as lipid-related markers, glycated hemoglobin, creatinine clearance, von Willebrand factor, and many others [60]. More recently, published data proposed CXCL-5 (epithelial neutrophil activating peptide-78) [61], soluble vascular adhesion protein-1 [62], glutamine, tyrosine, and docosahexaenoic acid [63] as markers of subclinical atherosclerosis. Additionally, our group recently used AOPP, IMA, and NO as markers of oxidative stress production and endothelial cell damage in disorders such as hypertriglyceridemia, hypercholesterolemia, and atherosclerosis [57, 64]. Observing the importance of these markers for the atherosclerosis progression, this review describe the significance of AOPP, IMA, and NO for early cardiovascular disease detection.

ADVANCED OXIDATION PROTEIN PRODUCTS

Proteins are continually under oxidative conditions in human tissues and body fluids, resulting in the formation of low levels of oxidized products [65]. A novel oxidative stress biomarker known as advanced oxidation protein products (AOPP) was discovered in 1996 and was first detected in the plasma of chronic uremic patients [66]. It has been suggested that AOPP is a measure of highly oxidized proteins in serum, particularly albumin. AOPP could be are derivatives of oxidation-modified albumin, which are formed under conditions of intensified oxidative stress, and quantitative measurement can provide information regarding the level of exposure to potentially damaging protein modifications, protein inactivation in ageing and disease, metabolic control, protein turnover, renal function, and other aspects of body function [65, 67]. Recent data have also indicated that oxidized fibrinogen is the key molecule responsible for the AOPP reaction in human plasma and that this process is involved in inflammation-associated events in atherosclerosis as well as in platelet aggregation [66, 68].

In this view, AOPP level in the serum is useful as a marker for monitoring the development of many disorders such as inflammation [69, 70], diabetes [67], and atherosclerosis [71] and can be helpful for monitoring of therapy used to treat these pathologies. Additionally, AOPP has been also suggested as a biomarker of oxidative stress and as a mediator of inflammation; AOPP may be involved in the conversion of vascular smooth muscle cells to osteoblasts, particularly in the presence of monocytes or macrophages, resulting in bone matrix protein formation [71]. Some studies have shown that increased arterial stiffness is associated with an increased risk of cardiovascular mortality and morbidity and that AOPP concentration is elevated in healthy people with increased stiffness index values [72].

Monocyte chemoattractant protein-1 is a chemokine that stimulates monocyte migration and plays a major role in the development of atherosclerosis [73]. Treatment of vascular smooth muscle cells in culture with AOPP resulted in a significant increase in the expression of monocyte chemoattractant protein-1 mRNA and protein, suggesting that AOPP contribute to the formation of atherosclerosis through pro-inflammatory mechanisms [74].

Common carotid artery intima-media thickness (CCA-IMT) has been accepted as a marker of the early atherosclerotic process (preclinical atherosclerosis) and has been associated with elevated cardiovascular disease risk [74, 75]. This marker is used to evaluate the presence of atherosclerosis and to assess the progression of this pathology [74, 76, 77]. Some studies have shown that increased circulating AOPP may be related to increased CCA-IMT [74, 78]. Yang *et al*. showed that patients with carotid artery plaques had significantly higher levels of AOPP and that their serum levels were strongly associated with carotid artery increased intima-media thickness and cross-sectional calculated intima-media area, suggesting that AOPP is involved in micro-inflammation pathogenesis, contributing to atherosclerosis development [78].

Recent works showed that oxidative stress can potentiate atherogenesis and this fact is related to the modification of biological structures and formation of new compounds as AOPP [79]. A study conducted by Kalousová *et al*. that evaluated the relationship between AOPP levels, atherosclerosis, and statin treatment revealed that patients with atherosclerosis have slightly higher levels of AOPP than healthy subjects [79]. Additionally, atherosclerosis patients treated with statins showed slightly lower AOPP levels compared to non-treated subjects, suggesting that alterations in AOPP levels are related to deregulation of lipid parameters. Another study revealed a relationship between elevated levels of AOPP, C-reactive protein, and fibrinogen and the development of coronary, cerebral, or peripheral artery occlusive accidents. This indicates that AOPP levels independently predict occlusive atherosclerotic cardiovascular events in patients with chronic kidney disease, contributing to accelerated atherogenesis in these patients [80]. Confirming previous results, hypercholesterolemic rabbits that received repeated intravenous injections AOPP showed more severe atherosclerotic lesions than did control groups, corroborating that AOPP accelerate the formation of atherosclerosis [81].

Modified fibrinogen has been also used as a cardiovascular disease risk biomarker [68], and various studies have demonstrated a relationship between AOPP levels and an increased risk of atherosclerosis [73, 74, 78, 79], suggesting that AOPP is useful as a preclinical marker for diagnosing this pathology. In addition, AOPP are formed in small quantities throughout life, but their levels increase with age [67]. However, under pathological conditions, higher concentrations of AOPP are observed, which was found to be an independent risk factor for coronary artery disease [78, 82], suggesting that AOPP is an important molecule for diagnosing preclinical atherosclerosis. However, despite evidence of the involvement of AOPP in atherosclerosis, as well as its potential contribution as a biomarker, few studies have explored its use in atherosclerosis research. Our study that was recently accepted for publication

revealed a direct relationship between increased levels of AOPP and patients with hypertriglyceridemia [64].

ISCHEMIA-MODIFIED ALBUMIN

IMA is also considered a promising biomarker of atherosclerotic conditions [34]. IMA is an *in vivo* modification product of human serum albumin by reactive oxygen species and has been proposed as a marker of diseases modulated by oxidative stress, such as chronic kidney disease [83], systemic sclerosis [84], hypercholesterolemia [85], and type II diabetes [86]. Decreased blood flow is related to oxidative stress production and these factors can alter the physiology of the arterial wall, playing an important role in atherosclerosis development [87]. Thus, IMA determination may be used to measure biomolecules damage *in vivo*, particularly in CVD or in subjects with risk factors for CVD development, and IMA is present in blood in easily detectable concentrations [57, 64].

Corroborating this hypothesis, patients with CAD (Coronary artery disease) have higher concentrations of IMA than controls [88]. Additionally, controls show higher total antioxidant capacity, which is inversely correlated with IMA levels. Interestingly, serum albumin was found to be decreased in these patients [88]. Albumin can act as an indirect antioxidant by binding free radicals. Low levels of albumin may be related to vascular diseases and atherosclerosis [89, 90]. Moreover, IMA serum concentration can provide earlier data of CAD installation before C-reactive protein and N-terminal-pro-brain natriuretic peptide [88].

Obesity is associated with the risk of developing CVD [91]. A study conducted in postmenopausal women revealed similar levels of IMA in women with proven CAD and overweight or obese women without CAD. However, both groups showed higher levels of IMA than non-obese women without CAD. This increase in IMA may be related to obesity-associated oxidative stress [91]. Interestingly, IMA has been correlated with being overweight, whereas other well-established markers such as C-reactive protein and N-terminal-pro-brain natriuretic peptide have not been associated with being overweight [91]. In agreement, IMA levels in hypercholesterolemic patients were not significantly correlated with other well-established atherosclerotic biomarkers, such as mass index, blood pleasure, lipid panels, and glucose [92]. Additionally, IMA was significantly higher and positively associated with HbA1c and homocysteine, while no correlation was found with ankle-brachial index in type II diabetes patients with peripheral arterial disease [93]. In contrast, patients have IMA levels similar to those of controls, while total antioxidant capacity is lower in slow coronary flow patients than in controls and is inversely correlated with IMA [94].

These results described above suggest that AOPP and IMA can be used as subclinical atherosclerosis biomarkers, contributing to early diagnosis of this pathology, particularly in subjects who do not show risk factors, reducing the morbidity and mortality related to disease development and progression.

NITRIC OXIDE

NO is known as a potent vasodilator and inhibitor of platelet adhesion and aggregation. In addition, NO can reduce leukocyte adherence to the vascular endothelium and suppress the proliferation of vascular smooth muscle cells by inhibiting growth factors. Recognizing the key enzymes in nitric oxide synthesis is important for understanding the pathophysiological mechanisms associated to NO in cardiovascular diseases, and these enzymes may be targets for new therapeutic interventions [95]. Nitric oxide synthases (NOSs) are a complex family of enzymes that catalyze the oxidation of L-arginine to form nitric oxide and L-citrulline. The three forms of human NOS identified thus far include endothelial constitutive (ecNOS), neuronal (nNOS), and inducible (iNOS). These enzymes are found on human chromosomes 7 (ecNOS), 12 (nNOS), and 17 (iNOS), and they were named based on the tissue in which were cloned and characterized first [96].

The isoform that is most involved in cardiovascular disease is ecNOS, as nearly all of its activity occurs in the heart, it is also present in the endothelium over the wide network of arteries, veins, and capillaries in the myocardium, as well in the endocardium of the heart chambers [97]. The role of NO in the regulation of vascular tone and platelet function is associated with the activity increase of ecNOS. The signal transduction pathway leading to activation of ecNOS in its entire course is regulated by an intracellular free calcium complex and calcium-calmodulin concentrations. Recent and detailed analyses of the association between ecNOS and the cell membrane showed that this enzyme is located in the Golgi apparatus, as well as specific structures in the membrane known as caveolae. The association between ecNOS and the plasma membrane region containing several concentrated signal transduction complexes (such as G proteins) likely has a large impact on enzyme activity, and also its accessibility to intracellular processes release via NO, including processes not associated with increases in intracellular calcium [98].

A study by Wilcox *et al*. evaluated human normal and atherosclerotic blood vessels by *in situ* hybridization and immunocytochemistry [100]. ecNOS was detected in endothelial cells of the normal human aorta, in fatty streaks, and in advanced atherosclerotic lesions. Comparison of the relative expression of ecNOS based on the von Willebrand factor in serial sections of normal and atherosclerotic vessels indicated that there was a decrease in endothelial cells expressing ecNOS in advanced atherosclerotic lesions. This event may lead to reduced NO production, moreover, NO inactivation limits the contribution of this molecule in blood vessel homeostasis and results in increased vascular tone and platelet adhesion and aggregation [98]. Thus, reduction in NO bioavailability is one of the main factors leading to endothelial dysfunction and consequently the development of CVD [98, 99]. Many studies have examined the pathophysiological mechanisms involved in this pathology, and have primarily focused on the role of NO [99].

In a review about the role of NO in atherosclerosis and hyperlipidemia, NO was described to have important physiological roles in vasodilation, vascular disease, and cytotoxicity. The NO and prostacyclin, both produced in the endothelium, act synergistically to inhibit platelet adhesion and aggregation. These autacoids also inhibit the adhesion and migration of leukocytes and have synergistic actions in terms of vasodilation in some arteries [102]. The development of atherosclerosis and hyperlipidemia is accompanied by endothelium-dependent vasodilation impairment. Additionally, atherosclerosis is associated with marked changes in the activity of NOS isoforms in the arterial wall, as well as demonstrated neointimal experimental animal models. The release of NO by the failure of normal physiological stimulation of endothelium, which has been attributed to defective ecNOS function, also creates favorable conditions for leukocyte adhesion, vasospasm, and thrombosis and can promote increased vascular intimal proliferation. Furthermore, NO and superoxide anions generated by inflammatory cells during atherosclerosis react to peroxynitrite radicals, potentially causing myocyte and endothelial injury; this effect may be a factor in cell apoptosis, leading to plaque rupture [102].

Concerning the effects of ecNOS on the expression of lipoprotein, molecular biological studies have indicated that the mRNA and protein ecNOS infrastructure are regulated by atherogenic levels of native low-density lipoprotein (nLDL) (level of 180 and 240 mg cholesterol/dL) after 48 hours of incubation with treated human umbilical vein endothelial cells, possibly at the transcriptional level. In addition, treatment of cells with high-density lipoproteins at human physiological concentrations (45 mg cholesterol/dL) did not appear to alter the expression of ecNOS, indicating that nLDL affects the rate of gene transcription by a mechanism that is dependent on concentration of nLDL. These findings may have significant

implications because they indicate the existence of a new mechanism whereby hypercholesterolemia induces early changes in endothelial cells that may have pathophysiologic significance in the atherosclerotic process [102].

The deposit of lipoproteins in the arterial wall, a key process in early atherogenesis, occurs at levels that are proportional to the concentration of these lipoproteins in the plasma [105]. In addition, strong evidence suggests that peroxidation of LDL components is crucial for the generation of atheroma [103]. A previous study showed that serum LDL (at normal levels) is encompassed in the endothelial cells through endocytosis mechanisms [104]. Inside cell lysosomes, the LDL particles encompassed are hydrolyzed in phospholipids, triglycerides (TG), cholesterol, and proteins. Finally, the specific receptors are recycled to the cell membrane surface. Free cholesterol is used in the formation of the cell membrane, in rearrangement, or stored in the form of cholesterol esters. Thus, approximately 10% of the particles reach the inner region of the blood vessel [104].

According to Flavahan, increased LDL in endothelial cells resulting from the normal process of endocytosis by specific and nonspecific receptors induces greater consumption of NO and marked production of free radicals [106]. The increase in free radicals leads to peroxidation of fatty acids of LDL particles, forming oxidized LDL. The oxidized LDL, through conversion of lecithin to lysolecithin, causes disruption of G proteins, which are membrane receptor agonists that act in numerous enhancers of NO production, leading to early endothelial dysfunction [106]. Some major effects of oxidized LDL include retention in the subendothelial space, recruitment and chemotaxis of monocytes/macrophages, activation of monocytes/macrophages and smooth muscle cells, which induce the intracellular uptake of LDL via the scavenger receptor to form "foam cells". This also promotes toxicity to endothelial cells, stimulating excessive production of superoxide radicals by these cells [107]. In addition, according to Fernández-Sánchez *et al*., these effects lead to excessive release of superoxide, contributing to its interaction with NO and subsequent formation of peroxynitrite, reducing NO levels [108].

Thus, the increase in oxidized LDL contributes to the initial change in endothelial cell function. Based on the results of previous studies, reduced NO level in atherosclerosis can be used as a marker for subclinical atherosclerosis. In support of this hypothesis, a study conducted by Proffumo *et al*. demonstrated that NO can be used as a marker for subclinical atherosclerosis in patients with autoimmune diseases, also it is associated with ROS [109]. However, a study by Santos *et al*., also in patients with autoimmune diseases, showed decreased NO in subjects with subclinical atherosclerosis, as assessed by ultrasound of the carotid artery [110]. The same study showed that the detection of NO levels in these patients may be complementary to imaging procedures as prognostic markers for the development of atherosclerotic disease and could be useful for the clinical management of patients.

RATIONALE FOR USING A NATURAL PRODUCT AS COMPLEMENTARY AND ALTERNATIVE MEDICINE

Integrative and complementary practices fall under the WHO determination; for traditional medicine and CAM, the WHO recommends that its member states develop national policies aimed the integration/inclusion of traditional medicine/CAM to official health systems, focusing on Primary Health Care [111, 112]. Thus, it is relevant the study of natural products that may be used for the control of cardiovascular disease, that together with the evaluation of subclinical biomarkers for atherosclerosis may be useful to reducing the risks for cardiovascular diseases.

Medicinal plants and their derivatives are among the main therapeutic resources of traditional medicine/CAM and have long been used by Brazilians for health care, whether in traditional/popular medicine or herbal medicine in public programs in the Health System, some for over 20 years. Among the Integrative and Complementary Practices in the Health System, medicinal plants and herbal medicine are widely used according to the Ministry of Health of Brazil, with most focused experiences for Primary Health Care [50].

The term phytotherapy was given to therapy that uses drugs whose active constituents are plants or plant products, and that has its origin in general knowledge and popular usage. The plants used for this purpose are traditionally referred to as medicinal plants [113]. Like in other healing cultures, traditional recipes are used preferably against chronic diseases, while serious or acute diseases are treated using Western medicine. The spread of traditional Chinese medicine to most of the other continents has contributed to the current popularity of herbal medicines worldwide. Examples of famous Chinese herbal medicines include *Angelica polymorpha var. sinensis* (Danggui, Dongquai), *Artemisia annua* (qing ha), *Paeonia lactiflora* (Bai shao yao), *Panax ginseng* (ren shen), and *Rheum palmatum* (da huang) [114, 115].

The magnitude of Brazilian biodiversity, which includes the set of all living beings with their full genetic variability, is not well-known, and it is estimated that there are more than two million different species of plants, animals, and micro-organisms. This fact places Brazil as having the greatest biological diversity in the world [116]. Despite this and the diversity of species, the potential use of plants as sources of new drugs has not been thoroughly examined, especially for cardiovascular diseases. This includes the pharmacological properties of plants, for which there have only been preliminary studies in many cases. For medical use, it is estimated that only 5 thousand species have been studied (RATES, 2001). In Brazil, which has approximately 55,000 species of plants, studies of only 0.4% of these flora have been reported [119].

The selection of plant species for study can be based on its traditional use in different societies as well as the chemical content and toxicity, selecting at random or by combining several criteria. The study of traditional medicine and/or popular treatments in different cultures is known as ethnopharmacology. Search strategies of drugs based on this line of work have been applied to treat different diseases [119, 120]. Medicinal plants, adopted by indigenous societies of oral tradition, may be useful in the development of phytochemicals and agronomics studies, preventing economic and time losses and demonstrating the importance of studying traditional medicinal plants commonly used in traditional societies [121, 122].

Many therapeutic agents available are used for the management of hypercholesterolemic individuals. A number of studies have demonstrated that lipid-lowering drugs can reduce the number of cardiovascular events and mortality from coronary disease [40]. Statins are effective for treatment and a group of drugs with similar chemical structures, whose common function is to competitively inhibit the enzyme 3-hydroxy-3-methyl-glutaryl coenzyme A (HMG-CoA) reductase. This enzyme catalyzes the conversion of HGM-CoA to mevalonate (steroid precursor), which is an essential step in cholesterol biosynthesis [123]. These drugs reduce 15-55% of LDL in adults, thereby reducing cardiovascular mortality and the incidence of acute coronary ischemic events. Additionally, the effect of statins extends beyond reducing lipid levels, known as a class pleiotropic effect, and these drugs exhibit anti-inflammatory effects by decreasing C-reactive protein levels and antioxidant effects by reducing reactive oxygen free radicals; for the latter, effects have been also observed for ischemic cardiac patients when cholesterol levels are within the normal range [124, 125].

Furthermore, the pleiotropic effects of statins have been increasingly recognized because of the anti-inflammatory and antioxidant properties of statins. Moreover, although statins are usually well-tolerated, there can be side effects, including dose-dependent elevation of transaminases in 0.5-2% of cases and nonspecific or muscle aches in joints without elevation of creatine (5%). In rare

cases, myositis-related muscle pain, elevation of creatine phosphokinase levels to 10 times the normal value (0.09% in clinical trials), hepatitis, and rhabdomyolysis can occur; the drugs also have a high cost and must be used for long periods [126].

Moreover, a diet restricting foods high in cholesterol as well as regular physical exercise should be proposed for the treatment of these individuals [42]. However, because of the resistance to dietary restrictions and financial limitations in the use of lipid-lowering drugs, many individuals have turned to alternative treatments to control cholesterol levels. Many of these alternative treatments have been used empirically and lack sufficient scientific support that allow for more reliable conclusions [43].

Different medicinal plants contain large amounts of antioxidants such as polyphenols, which play an important role in abstracting and neutralizing free radicals [131]. For example, grape seed polyphenols have a hypocholesterolemic effect in rats fed a cholesterol-rich diet [132]. In addition, supplementing drinking water with non-alcoholic wine, pomegranate juice, and quercetin reduces the size of lesions in mice deficient in apolipoprotein-E. These effects are associated with reduced uptake of LDL-cholesterol by macrophages and reduced susceptibility to LDL aggregation [133, 134]. In this view, there is continuing interest in the search for natural antioxidants for use in foods, cosmetics, or medicinal materials to replace synthetic antioxidants, which are sometimes restricted because of their carcinogenicity [127]. Antioxidant phytochemicals, particularly phenolic compounds found in vegetables, fruits, and medicinal plants, have received increasing attention for their potential roles in preventing human disease [128-130].

Interestingly, saponins have been found to have important biological activities, such as antifungal, antibacterial, anti-inflammatory, and hypocholesterolemic effects [135]. Saponins are natural detergents that form a heterogeneous group of triterpenes or steroid glycosides present in many plant species [136]. Several studies have shown that different types of saponins decrease serum cholesterol levels in animals and humans [137-139]. Afrose *et al*. showed that saponins were effective for reducing total cholesterol and LDL levels in rats fed a cholesterol-rich diet [140]. The hypocholesterolemic activity of saponins appears to occur in part by binding to cholesterol and bile acids in the gut lumen, thereby increasing the excretion of these steroids in the feces [136]. As a result, cholesterol metabolism is accelerated and cholesterol levels are decreased.

CAMPOMANESIA XANTHOCARPA AS CAM FOR ATHEROSCLEROSIS TREATMENT

Interestingly, the use of teas as dietary supplements arises from the information that some compounds present in this drink have beneficial protective effects against chronic diseases [141]. There is a growing use of plants as CAM in worldwide. However, there are few studies of Brazilian native plants that really demonstrate effectiveness in reducing cholesterol levels as has been done with *Campomanesia xanthocarpa* Berg. (Myrtaceae) by our group. Popular accounts about the traditional use of tea from *C. xanthocarpa*, commonly known as "guavirova", present in the South of Brazil but also found in Argentina, Paraguay, and Uruguay [54], have indicated its use for several diseases, including inflammatory diseases and hypercholesterolemia [55]. According to a study of plant sellers in Porto Alegre (Brazil), *C. xanthocarpa* is empirically used for weight reduction and its potential effect on controlling a number of conditions associated with obesity, including hyperlipidemia [43].

Recent studies by our research group showed a lipid-lowering effect of guavirova, as it prodeuced a promoted reduction in serum LDL-cholesterol and inhibited the activity of HMG-reductase enzyme, had antioxidant effects by reduction of carbonyl protein, and prevented the production of hydrogen peroxide [56]. Recently, our group confirmed the effects of the plant in a study by Viecili *et al*.; the hypocholesterolemic effect was verified in a larger series of hypercholesterolemic individuals, the plant even showed antioxidant and anti-inflammatory effects and improved the availability of NO in these individuals [57]. Interestingly, these effects were similar to the effects of statins, which also showed lipid-lowering, anti-inflammatory, and antioxidant effects [142]. The group also demonstrated that guavirova has antiplatelet, antithrombotic, and fibrinolytic activities in mice, showing no ulcerogenic activity in oral administration compared to acetylsalicylic acid [58].

In order to clarify which of the components were responsible for the lipid-lowering effect, the group examined the leaves of *C. xanthocarpa* by high-performance liquid chromatography using a diode array detector (HPLC-DAD) to detect the following most abundant components of the plant: gallic acid, chlorogenic acid, rutin, quercetin, and kaempferol (preliminary results). Based on the results, it was evident that among the components found in the leaf, only gallic acid and chlorogenic acid had mechanisms similar to the lipid-lowering effect of statins. These compounds concentration-dependently inhibited enzyme activity as a limiting step in cholesterol synthesis by HMG-CoA reductase (preliminary results).

Based on a study of the components identified by HPLC-DAD, it was found that gallic acid and chlorogenic acid, both phenolic acids, perform efficiently and similarly to pravastatin, and its concentration-dependently inhibiting the activity of HMG-CoA reductase enzyme. Plant-derived polyphenolic compounds have a wide range of pharmacological properties, and their mechanisms of action have been the subject of considerable interest in recent years. This finding is consistent with the results of Punithavathi *et al*., who demonstrated that mice with streptozotocin-induced diabetes have increased hepatic HMG-CoA reductase activity [143], which was previously reported by Catanzaro and Suen [144]. This activity was assessed in a liver homogenate, and treatment with gallic acid (10 and 20 mg/kg) reduced enzyme activity and cholesterol levels in mouse livers. Therefore, this inhibitory activity is very similar to that found preliminarily by our research group, as gallic acid inhibited the HMG-CoA reductase enzyme, which has been verified using assays involving the purified human catalytic subunit of the enzyme purified.

Notably, chlorogenic acid also strongly inhibited HMG-CoA reductase according to Carvalho [145]. This phenolic acid is a major polyphenol present in plants, fruits, and vegetables [146] and showed the capacity to reduce cardiovascular risk and decrease the oxidation of LDL and total cholesterol [147]. Supporting the effect of chlorogenic acid, a study by Cho *et al*. demonstrated that this compound reduces fatty acid synthesis and cholesterol by inhibiting HMG-CoA reductase [148]. Accordingly, chlorogenic acid is thought to be involved in the inhibition of HMG-CoA reductase from *C. xanthocarpa.*

The anti-HMG-CoA reductase activity observed in *C. xanthocarpa* may also have been caused by synergistic effect of gallic acid and chlorogenic acid, and it is possible that other components present may be involved in enzyme inhibition. Corroborating this result, a study by Chang *et al*. demonstrated that tannins, which are also present in plants, can inhibit HMG-CoA reductase [149].

Based on the results of previous studies examining guavirova, it can be concluded that this plant has beneficial medical effects, acting as a lipid-lowering, anti-inflammatory, and antioxidant factor that improves endothelial conditions. This suggests that the plant has pleiotropic effects that are similar to those of statins and has lipid-lowering properties [125]. Thus, efficient trajectory that involves the study of guavirova by our research group allows us to best deepen of the studies, especially as regards the improvement of the subclinical atherosclerosis with natural products.

Clinical evidence indicates that *C. xanthocarpa* reduced inflammatory responses associated with the reduction of C-reactive protein, which improved endothelial dysfunction by increasing NO levels; there is also an increase in the anti-oxidant effect with the

reduction of IMA and AOPPs levels [57]. The results of a study conducted by our group confirmed the ethnopharmacological use of *C. xanthocarpa* and demonstrated that the treatment reduced TC and LDL levels in hypercholesterolemic individuals [57].

Thereby, *C. xanthocarpa* may provide an alternative treatment option for hypercholesterolemic individuals, as the use of oral hypolipemiants is limited because of their adverse effects [126]. Furthermore, *C. xanthocarpa* reduced LDL levels to the amount required to affect the size of atherosclerotic plaques [150]. Our results of examination of hypercholesterolemic individuals agree with the results of our previous preliminary clinical study in which we found that *C. xanthocarpa* reduced plasma cholesterol and LDL levels in individuals with hypercholesterolemia [56]. Thus, the study made important contributions to the data available for *C. xanthocarpa*, as few studies have focused on the lipid profile in humans.

In addition, hypercholesterolemia and an increased inflammatory response are associated with higher levels of oxidative stress, which is an important event in the development and maintenance of atherosclerosis [151]. Although an increase in oxidative stress and inflammation in hypercholesterolemia was previously reported, the study of a plant by our group was the first to report a reduction in AOPPs (maximum reduction of 58 ± 6%); AOPPs are biomarkers of protein oxidation and comprise a new class of inflammatory mediators in hypercholesterolemic subjects [57]. AOPPs are excellent markers of oxidative stress, and their role in the development of cardiovascular disease may be very important [152]. Previous studies have demonstrated that the association between AOPPs and hypercholesterolemia was independent of age, sex, smoking, body mass index, and waist circumference [153]. Our results showed that the consumption of *C. xanthocarpa* for 90 days reduced the levels of AOPPs in hypercholesterolemic individuals [57]. Further, we found a positive correlation between high cholesterol or triglyceride levels and AOPP production ($r = 0.14$, $P = 0.04$ and $r = 0.79$, $P < 0.0001$) [64], which was in agreement with the results of previous studies [153, 154].

The incidence of atherosclerosis increases with hypercholesterolemia, and oxidative stress plays a key role in the pathogenesis and development of this pathology [155]; its products may be useful as disease progression markers and have been the focus of biomedical research. Increased oxidative stress by products and/or a reduced antioxidant activity are the main causes of atherosclerosis and endothelial dysfunction [156]. As previously reported, overproduction of free radicals may chemically modify human serum albumin, resulting in increased IMA; IMA appears to function as an oxidative stress biomarker [85]. In our study, we observed a significant decrease in IMA levels in hypercholesterolemic subjects (maximum reduction of 24 ± 6%) [57]. Moreover, a positive correlation between high cholesterol or triglyceride levels and IMA production was detected by our group [64], which agrees with previous results [85]. Measuring IMA in the serum of asymptomatic hypercholesterolemia subjects treated with *C. xanthocarpa* may be of prognostic benefit, suggesting an improvement in IMA in people with possible subclinical atherosclerotic lesions.

Free radicals play an important role in endothelium dysfunction, which is characterized by functional disruption of the protective endothelium, releasing internalized cholesterol as well as causing the recruitment of inflammatory cells into the vessel wall and initiating the atherosclerotic process [157]. Alterations in NOx synthesis or its physiological activity can play a central role in endothelial dysfunction [107, 158]. NO plays a protective role by suppressing the abnormal proliferation of vascular smooth muscle cells in various pathological situations, including atherosclerosis [107]. Previous studies demonstrated that hypercholesterolemic subjects showed lower levels of NO [153]. Decreased production of NO in hypercholesterolemia causes serious disruptions in endothelial equilibrium [159]. In our study, we found that administration of *C. xanthocarpa* was associated with a significant increase in NO in hypercholesterolemic individuals after 90 days (maximum reduction of 127 ± 69%) of treatment [57].

In summary, the effect of *C. xanthocarpa* appears to be similar to the pleiotropic effect of statins, where the vasculoprotective effect of statins is mainly mediated through the inhibition of the mevalonate pathway and oxidized LDL generation, which enhances the biosynthesis of endothelium-derived NO [160]. We suggest that administration of *C. xanthocarpa*, which acts as a statin to inhibit HMG-CoA reductase [56], attenuates the oxidative stress and proinflammatory reactions as well as enhances blood flow by improving endothelial function. Finally, our results provided the first clinical evidence in humans as well as encouraging results that point to a protective effect of *C. xanthocarpa* on subclinical atherosclerosis, aside from that the plant can also be used to protect healthy individuals.

CONCLUSION

The search for new biomarkers for subclinical atherosclerosis has shown advancement towards new discoveries useful for treating subclinical atherosclerotic processes. However, for routine use in the clinic, additional studies are needed to standardize its accuracy (sensitivity, specificity, predictive values). Moreover, these biomarkers must be available at a low cost and accessible to all levels of healthcare, particularly individuals with subclinical disease. Then the evaluation of AOPP, IMA and NO levels could help to improve the detection of the subclinical cardiovascular risk.

CAM is currently advancing with increasing studies involving plants and natural products. Some of these products can act in various mechanisms of subclinical atherosclerosis as antioxidants and have anti-inflammatory, lipid-lowering, and hypoglycemic activities. The use of plants and natural products can be used to reduce protein oxidation and improve the availability of NO and thus vascular function. Few plants and natural product flora have been studied, and the vast majority must still be examined to drive the improvement of healthcare and reduce the costs for governments. One of these could be *C. xanthocarpa*, which presents encouraging results for use in the recovery and maintenance of the cardiovascular health.

LIST OF ABBREVIATIONS

CVD	=	Cardiovascular Disease
MI	=	Myocardial Infarction
IMA	=	Ischemia-modified albumin
AOPP	=	Advanced oxidation protein products
WHO	=	World Health Organization
ROS	=	Reative oxygen Species
NO	=	Nitric Oxide
CAM	=	Complementary and alternative medicine
CXCL-5	=	epithelial neutrophil activating peptide-78
CCA-IMT	=	Common carotid artery intima-media thickness
CAD	=	Coronary artery disease
NOSs	=	Nitric oxide synthases
ecNOS	=	Nitric oxide synthase constitutive
nNOS	=	Nitric oxide synthase neuronal
iNOS	=	Nitric oxide synthases inducible
nLDL	=	Native low-density lipoprotein
LDL	=	Low-density lipoprotein
HMG-CoA	=	3-hidroxi-3-methil glutaril CoA
HPLC-DAD	=	High performance liquid chromatography with diode array detection
TC	=	Total cholesterol

TG = Triglycerides

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

This study was supported by the Instituto de Cardiologia de Cruz Alta (ICCA). Fellowships from the Programa Institucional de Bolsas de Iniciação Científica - (PIBIC) - Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) are also acknowledged.

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Received: August 1, 2015 **Accepted: November 11, 2015**

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