UNIVERSIDADE FEDERAL DO PAMPA

BRUNA COCCO PILAR

EFEITOS DO ÓLEO DE LINHAÇA E DA LIGNANA SECOISOLARICIRESINOL DIGLICOSÍDEO (SDG) EM UM MODELO DE SÍNDROME METABÓLICA EM RATOS WISTAR

TESE DE DOUTORADO

Uruguaiana
2017

BRUNA COCCO PILAR

EFEITOS DO ÓLEO DE LINHAÇA E DA LIGNANA SECOISOLARICIRESINOL DIGLICOSÍDEO (SDG) EM UM MODELO DE SÍNDROME METABÓLICA EM RATOS WISTAR

Tese apresentada ao programa de Pós-graduação *Stricto Sensu* em Bioquímica da Universidade Federal do Pampa, como requisito parcial para obtenção do Título de Doutora em Bioquímica.

Orientadora: Profª. Drª. Vanusa Manfredini

Uruguaiana
2017

BRUNA COCCO PILAR

EFEITOS DO ÓLEO DE LINHAÇA E DA LIGNANA SECOISOLARICIRESINOL DIGLICOSÍDEO (SDG) EM UM MODELO DE SÍNDROME METABÓLICA EM RATOS WISTAR

Tese apresentada ao programa de Pós-graduação *Stricto Sensu* em Bioquímica da Universidade Federal do Pampa, como requisito parcial para obtenção do Título de Doutora em Bioquímica.

Tese defendida e aprovada em: 24 de novembro de 2017.

Banca examinadora:

Profª. Drª. Vanusa Manfredini
Orientadora
(UNIPAMPA)

Profª. Drª. Cristiane Casagrande Denardin
(UNIPAMPA)

Profª. Drª. Francielli Weber Santos Cibin
(UNIPAMPA)

Prof. Dr. Michel Mansur Machado
(UNIPAMPA)

Profª. Drª. Cristiane Matté
(UFRGS)

AGRADECIMENTO

Agradeço, em primeiro lugar, a Deus por me guiar, iluminar e me dar tranquilidade para seguir em frente com os meus objetivos e não desanimar com as dificuldades.

À N. Sr.ª Aparecida por me iluminar nos momentos mais difíceis dessa trajetória. Serei-lhe eternamente fiel!

Aos meus pais, Vera e Vanderlei, principais responsáveis pela minha vida e a quem devo meu caráter e disciplina ao trabalho! Obrigada pelo incentivo, amor e amparo em todos os momentos.

Ao Tiago, pelo amor, carinho e apoio. Obrigada pela compreensão e incentivo para seguir em frente. Te amo!

Ao meu irmão que, mesmo de longe, sempre me apoiou e acreditou no meu potencial.

Aos amigos que, fora do laboratório, me acompanharam neste percurso. Obrigada pelas palavras de incentivo e pelos momentos de descontração.

Aos colegas de trabalho que sempre estiveram ao meu lado para me escutar durante os momentos difíceis e compartilhar comigo os momentos de alegria! Muito obrigada pelo apoio e pelos pensamentos positivos!

Agradeço imensamente à UNIPAMPA e ao Programa de Pós-Graduação em Bioquímica pela oportunidade de me tornar Doutora sem me afastar de meus familiares.

Um agradecimento especial à minha orientadora profª. Vanusa por ser minha primeira guia no mundo da pesquisa e da pós-graduação. Muito obrigada por todo o apoio, ensinamentos e amizade!

Aos colegas do grupo GESTOX, que me acolheram e colaboraram em todas as etapas do desenvolvimento deste trabalho.

Ao Laboratório de Estudos Físico-Químicos e Produtos Naturais da UNIPAMPA, especialmente ao professor Elton e ao colega Jefferson pela disponibilidade na análise do óleo de linhaça.

Ao Laboratório de Pesquisa em Fisiologia Cardiovascular da UNIPAMPA, especialmente à profª. Giulia e à Aline pelo empréstimo e auxílio na utilização do equipamento de pletismografia.

Ao Laboratório de Análise de Metabólitos do Serviço de Genética do HCPA pela disponibilidade na análise dos parâmetros inflamatórios e das enzimas antioxidantes.

A todos os professores do PPG-Bioquímica por tudo que me ensinaram com suas aulas e mesmo fora delas durante esses anos.

RESUMO

Evidências têm demonstrado um papel positivo da semente de linhaça no tratamento e prevenção da síndrome metabólica (SM) e das complicações associadas à mesma. No entanto, não há um consenso sobre o componente responsável por esses efeitos. Para esclarecer essa questão, o objetivo do presente estudo foi avaliar os efeitos do óleo de linhaça e da lignana da linhaça secoisolariciresinol diglicosídeo (SDG) em um modelo de síndrome metabólica em ratos Wistar. Para isso, 48 ratos Wistar foram distribuídos em seis grupos (n=8): Os grupos I (control), V (FO) e VI (SDG) receberam água *ad libitum*, e os grupos II (MS), III (MS + FO) e IV (MS + SDG) receberam 30% de frutose na água de beber para indução da SM. Concomitantemente, os animais receberam solução salina (grupos I e II), óleo de linhaça (grupos III e V) e SDG (grupos IV e VI) por via oral. Após 30 dias, os animais foram sacrificados, sendo o sangue, o fígado e os rins coletados para análises bioquímicas, oxidativas, inflamatórias e histopatológicas. O peso corporal foi registrado semanalmente. A pressão arterial sistólica foi medida antes e após o tratamento. Foi realizada análise do óleo de linhaça por cromatografia gasosa acoplada a espectrometria de massas, a qual indicou a presença predominante de ácidos graxos, esteróides e tocoferóis. A solução de frutose foi capaz de induzir SM, além de promover alterações oxidativas, inflamatórias e renais. O óleo de linhaça e o SDG preveniram o desenvolvimento de SM, bem como as alterações oxidativas, inflamatórias e renais. Assim, os resultados deste estudo demonstraram que o tratamento com solução de frutose a 30% durante 30 dias é um modelo eficaz para indução de SM em ratos Wistar. Além disso, a suplementação com óleo de linhaça e SDG foi capaz de prevenir de maneira semelhante as alterações pressóricas, oxidativas, bioquímicas e inflamatórias observadas neste transtorno, o que demonstrou que ambos podem ser utilizados na prevenção do desenvolvimento de SM e de suas complicações em ratos.

Palavras-chave: Óleo de linhaça. Secoisolariciresinol diglicosídeo. Síndrome metabólica.

ABSTRACT

Evidence has shown a positive role for flaxseed in the treatment and prevention of metabolic syndrome (MS) and the associated complications. However, there is no consensus on the component responsible for such effects. To clarify this issue, the objective of the present study was to evaluate the effects of flaxseed oil and flaxseed lignan secoisolariciresinol diglycoside (SDG) in a metabolic syndrome model in Wistar rats. Forty-eight Wistar rats were allocated into six groups (n = 8): Groups I (control), V (FO) and VI (SDG) received water *ad libitum*, and groups II (MS), III (MS + FO) and IV (MS + SDG) received 30% fructose in drinking water for MS induction. Concomitantly, the animals received saline (groups I and II), flaxseed oil (groups III and V) and SDG (groups IV and VI) orally. After 30 days, the animals were sacrificed, and blood, liver and kidneys were collected for biochemical, oxidative, inflammatory and histopathological analyzes. Body weight was recorded weekly. Systolic blood pressure was measured before and after treatment. Flaxseed oil analysis was performed by gas chromatography-mass spectrometry, which indicated the predominant presence of fatty acids, steroids and tocopherols. Fructose solution induced MS, besides promoting oxidative, inflammatory and renal changes. Flaxeed oil and SDG prevented the MS development as well as oxidative, inflammatory and renal changes. Thus, the results of this study demonstrated that treatment with 30% fructose solution for 30 days is an effective model for MS induction in Wistar rats. In addition, supplementation with flaxseed oil and SDG prevented similarly the pressure, oxidative, biochemical and inflammatory changes observed in this disorder, which demonstrated that both can be used to prevent the MS development and its complications in rats.

Keywords: Flaxseed oil. Secoisolariciresinol diglycoside. Metabolic syndrome

LISTA DE FIGURAS

Figura 1 – *Linum usitatissimum* L. (linho) 17
Figura 2 – Sementes de linhaça dourada e marrom 18
Figura 3 – Estrutura do ácido α-linolênico (ALA) 19
Figura 4 – Estrutura do secoisolariciresinol diglicosídeo (SDG) 21

ARTIGO

Figure 1 - Body weight gain of rats during the experimental period 34
Figure 2 - Systolic blood pressure in different study groups before and after 30 days of FO supplementation 34
Figure 3 - Biomarkers of oxidative damage in different study groups 35
Figure 4 - Non-enzymatic antioxidant levels in different study groups 36
Figure 5 - Enzymatic antioxidant defenses in different study groups 37

MANUSCRITO

Figure 1 - Percent increase in systolic blood pressure during the experimental period. 60
Figure 2 - Histopathological sections of livers. 60
Figure 3 - Histopathological sections of kidneys. 61

LISTA DE TABELAS

Tabela 1 – Componentes da Síndrome Metabólica segundo o NCEP-ATP III..... 24

ARTIGO

Table 1 - Biochemical parameters in different study groups..... 35

MANUSCRITO

Table 1 - Biochemical and inflammatory parameters in different study groups..... 49

LISTA DE ABREVIATURAS E SIGLAS

ALA – Ácido α-linolênico

AGL – Ácidos graxos livres

AGPI – Ácidos graxos poliinsaturados

ANVISA – Agência Nacional de Vigilância Sanitária

AT1 – Receptor de angiotensina tipo 1

CAT - Catalase

CCl_4 – Tetracloreto de carbono

DCV – Doenças cardiovasculares

DHA – Ácido docohexaenóico

DM2 – Diabete Mellitus tipo 2

DNA – Ácido desoxirribonucleico

ED – Enterodiol

EL – Enterolactona

EPA – Ácido eeicosapentanóico

ERO – Espécies reativas de oxigênio

FID – Federação Internacional de Diabetes

GLUT 5 – Transportador de glicose tipo 5

GPx – Glutationa peroxidase

GR – Glutationa redutase

GSH – Glutationa reduzida

GST – Glutationa-S-transferase

HDL – Lipoproteína de alta densidade

I-DBSM – I Diretriz Brasileira de Diagóstico e Tratamento da Síndrome Metabólica

IBGE – Instituto Brasileiro de Geografia e Estatística

IL-6 – Interleucina 6

IMC – Índice de massa corporal

LDL – Lipoproteína de baixa densidade

NCEP-ATP III – *National Cholesterol Education Program Adult Treatment Panel III*

OMS – Organização Mundial da Saúde

PAI-1 – Inibidor da ativador de plasminogênio

SDG – Secoisolariciresinol diglicosídeo

SECO – Secoisolariciresinol

SM – Síndrome metabólica

SOD – Superóxido dismutase

TNF-α – Fator de necrose tumoral α

VI-DBH – VI Diretrizes Brasileiras de Hipertensão

VLDL – Lipoproteína de muito baixa densidade

SUMÁRIO

1 INTRODUÇÃO 13
2 CONCEITOS GERAIS E REVISÃO DE LITERATURA 15
2.1 Alimentos Funcionais e compostos bioativos 15
2.2 Linhaça (*Linum usitatissimum* L.) 16
2.2.1 Origem 16
2.2.2 Caracterização 17
2.2.3 Composição Nutricional 18
2.2.3.1 Proteínas vegetais 18
2.2.3.2 Fibras alimentares 19
2.2.3.3 Óleo de linhaça e ácidos graxos poli-insaturados 19
2.2.3.4 Compostos fenólicos 20
2.2.4 Atividades biológicas descritas 21
2.3 Síndrome Metabólica 23
2.3.1 Origem 23
2.3.2 Critérios para o diagnóstico 23
2.3.3 Fisiopatologia 25
2.3.4 Tratamento 26
2.3.5 Modelos animais 28
3 OBJETIVOS 30
3.1 Objetivo Geral 30
3.2 Objetivos específicos 30
4 ARTIGO 31
5 MANUSCRITO 40
6 CONCLUSÃO 62
REFERÊNCIAS 63
ANEXO A - Parecer de aprovação do Comitê de Ética no Uso de Animais (CEUA) da UNIPAMPA 71

APRESENTAÇÃO

A presente tese foi dividida em três partes principais. Na **parte I** encontram-se a **INTRODUÇÃO,** os **CONCEITOS GERAIS E REVISÃO DA LITERATURA** e os **OBJETIVOS.** As seções **MATERIAL E MÉTODOS, RESULTADOS** e **DISCUSSÃO** e as respectivas **REFERÊNCIAS** estão apresentadas sob a forma de um artigo e um manuscrito, os quais compõem a **parte II** deste trabalho e representam a íntegra deste estudo. As seções **CONCLUSÃO** e **REFERÊNCIAS** encontram-se na **parte III** desta tese, sendo que as referências referem-se somente às citações utilizadas na introdução e nos conceitos gerais e revisão da literatura da mesma.

PARTE I

1 INTRODUÇÃO

A crescente compreensão da relação entre a dieta e a saúde tem levado os consumidores a uma tendência para o consumo de alimentos nutritivos com funções adicionais de promoção da saúde no tratamento e prevenção de doenças, os quais são chamados de alimentos funcionais (OLMEDILLA-ALONSO et al.,2013). Estes alimentos apresentam, em sua maioria, compostos como ácidos graxos poli-insaturados (AGPI), fibras dietéticas, vitaminas e polifenóis, os quais são bastante conhecidos por reduzir lipídios e açúcares sanguíneos além de apresentarem, entre outras, atividade antioxidante (ANWAR & PRZYBYLSKI, 2012).

A Síndrome Metabólica (SM) é caracterizada por um conjunto de alterações metabólicas, com alto custo sócio-econômico e que é considerada uma epidemia global. Este distúrbio é definido por um conjunto de fatores interligados que aumentam o risco de DCV e diabetes mellitus tipo 2 (DM2) (KASSI et al., 2011). Seus principais componentes são a obesidade abdominal, a dislipidemia, a hipertensão, a hiperglicemia e a resistência à insulina, os quais contribuem para o aumento da morbidade e mortalidade cardiovascular (NAGAO & YANAGITA, 2010). Além disso, estudos já demonstraram que a SM está associada a um estado pró-inflamatório, além de aumentar o risco de aparecimento de doenças hepáticas e renais (GLUBA et al., 2012; VOS & LAVINE, 2013; KRAJA et al., 2014).

O tratamento da SM é complexo e inclui tanto mudanças no estilo de vida quanto tratamento medicamentoso (GRUNDY et al., 2004), sendo que muitos estudos indicam que o aumento da ingestão de alimentos funcionais e suplementos antioxidantes está inversamente relacionado com a presença dos fatores de risco associados à SM (FERNANDES et al., 2007; PATTYN et al., 2013; PILAR et al, 2014).

A semente de linhaça (*Linum usitatissimum* L.) é um alimento funcional que tem despertado crescente interesse dos pesquisadores por conter componentes biologicamente ativos, tais como fibras dietéticas, proteínas vegetais, ácidos graxos poli-insaturados (AGPI) e lignanas, que desempenham funções benéficas no organismo, possibilitando a prevenção de diversas doenças (RUBILAR et al., 2010; TAYLOR et al., 2010; KAJLA et al., 2015).

A semente de linhaça apresenta aproximadamente 57% de ácido alfa-linolênico (ALA) quando na forma de óleo, sendo uma das fontes mais ricas de ALA. Já foi demonstrado em

estudos anteriores que o ácido eicosapentaenóico (EPA) e o ácido docosahexanóico (DHA), que são formados no organismo a partir do ALA, reduzem o risco de muitas doenças crônicas, como a aterosclerose, as doenças cardiovasculares (DCV), o câncer e a hiperlipidemia. Além disso, a semente de linhaça é rica em lignanas, fitoestrógenos comumente consumidos na dieta humana, sendo o secoisolariciresinol diglicosídeo (SDG) a principal lignana encontrada (cerca de 1% do seu peso seco) (KHALESI et al., 2011). Após seu metabolismo no intestino e cólon, o SDG é quebrado em moléculas de secoisolariciesinol (SECO), enterodiol (ED) e enterolactona (EL). Estudos já demonstraram efeitos benéficos desses metabólitos na redução do estresse oxidativo e dos níveis de lipídios sanguíneos (HU et al., 2007; ZHANG et al., 2008).

No entanto, apesar da semente de linhaça já ter apresentado efeitos benéficos para a saúde, inclusive em pacientes com SM, ainda existe uma lacuna quanto ao componente responsável por tais efeitos, visto que alguns autores atribuem aos AGPI (como o ALA) enquanto outros atribuem às lignanas (especialmente ao SDG). Dessa forma, este estudo se propõe a comparar os efeitos do óleo de linhaça e da lignana SDG na prevenção das alterações pressóricas, bioquímicas, inflamatórias e de estresse oxidativo associadas à SM em ratos.

2 CONCEITOS GERAIS E REVISÃO DE LITERATURA

2.1 Alimentos Funcionais e compostos bioativos

A crescente compreensão da relação entre a dieta, os ingredientes alimentares específicos e a saúde está levando a novas ideias sobre o efeito dos componentes dos alimentos sobre a função fisiológica e a saúde. Atualmente, os alimentos não são destinados apenas para saciar a fome e fornecer os nutrientes necessários para os seres humanos, mas também para prevenir doenças relacionadas com a nutrição e melhorar o bem-estar físico e mental dos consumidores (SIRÓ et al., 2008). Além disso, as pessoas têm se tornado mais conscientes com relação à saúde, conduzindo a uma tendência para o consumo desses alimentos nutritivos e com funções adicionais de promoção à saúde, os quais são chamados de alimentos funcionais (OLMEDILLA-ALONSO et al.,2013).

O termo " alimento funcional " em si foi usado pela primeira vez no Japão, na década de 1980, através de um programa de governo que tinha como objetivo desenvolver alimentos saudáveis para uma população que envelhecia e apresentava uma grande expectativa de vida (MORAES & COLLA, 2006). Hoje, o Japão já possui um sistema de regulamentação bem definido e estabelecido e, seguindo seu exemplo, outros países da Ásia conseguiram desenvolver regulamentos para a produção e utilização de alimentos funcionais (ZAWISTOWSKI, 2008). Na Europa, um alimento funcional é aquele no qual foram demonstrados benefícios a uma ou mais funções alvo do corpo, além dos efeitos nutricionais, de forma a contribuir para melhorar o estado de saúde, bem-estar e/ou redução do risco de doenças (GULATI & OTTAWAY, 2008).

Na América Latina, o Brasil é o único país que possui uma legislação que inclui alegações funcionais e de saúde para componentes nutrientes ou não-nutrientes. Embora não haja uma definição oficial para o termo “alimentos funcionais” no Brasil, as normas são baseadas no conceito de que são alimentos e não drogas, e dessa forma fazem parte de uma dieta normal com efeitos benéficos à saúde (SOUZA, 2014). A Agência Nacional de Vigilância Sanitária (ANVISA) regulamentou por meio da Resolução nº 18, de 30 de abril de 1999 a definição de propriedades funcionais, na qual consta o papel metabólico ou fisiológico que o nutriente ou não nutriente tem no crescimento, desenvolvimento, manutenção e outras funções normais do organismo humano (BRASIL, 1999). Os principais alimentos incluídos neste grupo são os de origem vegetal, como frutas, vegetais e grãos integrais, assim como os alimentos marinhos e seus ácidos graxos de cadeia longa (SHAHIDI, 2009).

Além disso, a legislação brasileira também reconhece a nomenclatura de compostos bioativos, através da RDC nº 2, a qual traz as diretrizes a serem adotadas para avaliação de segurança, registro e comercialização de substâncias bioativas e probióticos isolados com alegações de propriedade funcional e/ou para a saúde. Estes produtos, entre outros, incluem os carotenoides, os flavonóides e os polifenóis (BRASIL, 2002).

2.2 Linhaça (*Linum usitatissimum* L.)

2.2.1 Origem

Os relatos mais antigos da semente de linhaça são datados de 5000 anos antes de Cristo, na Mesopotâmia. Até a década de 1990, a linhaça era utilizada principalmente para a confecção de panos e papéis, no entanto seus benefícios nutricionais foram difundidos pelo mundo todo, sendo seu consumo muito comum na América do Norte e em países europeus (MONEGO, 2009). Hoje, a linhaça é cultivada em mais de 2,6 milhões de hectares e os mais importantes países produtores de linho são o Canadá, a Índia, a China, os Estados Unidos e a Etiópia. O Canadá, com 840.000 metros produzidos nos anos 2015-2016, é o maior produtor mundial de linhaça e representa cerca de 80% do comércio global da mesma (BERNACCHIA et al., 2014; FLAX COUNCIL OF CANADA, 2017).

Na América do Sul, a maior produção ocorre na Argentina e no Brasil, sendo que neste último a linhaça foi introduzida no século XVII em Santa Catarina. Atualmente, a maior produção de linhaça do Brasil ocorre no noroeste do Rio Grande do Sul, principalmente nas cidades de Ijuí, Tupanciretã, São Miguel das Missões, São Luiz Gonzaga, Giruá, Santa Rosa, Guarani das Missões, Três de Maio, Panambi, Santa Bárbara do Sul, Santo Augusto e proximidades. Nesta região, a produção de linhaça vem apresentando um aumento de aproximadamente 20% nos últimos anos devido à grande demanda pelas indústrias de alimentos e como alternativa de produção no inverno. No entanto, grande parte da linhaça consumida no Brasil, ainda é importada do Canadá, Estados Unidos, China e Argentina (MARQUES, 2008; REDLICH, 2016).

Segundo o relatório de 2016 do Instituto Brasileiro de Geografia e Estatística (IBGE) sobre a produção agrícola municipal, no Brasil foram produzidas 12.973 toneladas desta semente, com um rendimento de 1.113 kg/ha. Com estes dados ficou registrado um aumento produtivo de 6% em relação a 2015 (IBGE, 2016).

2.2.2 Caracterização

O linho é uma planta pertencente à família Linaceae, tendo como nome científico *Linum usitatissimum*. Esta planta é caracterizada por apresentar uma altura de 30 a 130 cm, talos eretos, folhas estreitas lineares ou lanceoladas, alternando entre o verde e o verde claro, além de flores com pétalas azuis (Figura 1) (MARQUES, 2008). Da casca da planta (caule) é retirada a fibra do linho, utilizada para a fabricação de tecidos, e da cápsula se obtém a semente, chamada de linhaça (HALATENO, 2016).

Figura 1 – *Linum usitatissimum* L. (linho).

Fonte: https://fairdinkumseeds.com/products-page/flowers-and-ornamentals/linseed-linen-linum-usitatissimum-seeds/, acessado em 08/05/2017.

A semente de linhaça é plana e oval com uma extremidade afunilada. É um pouco maior do que a semente de gergelim, tendo entre 4 e 6 mm. A cor das sementes pode variar do marrom escuro para o dourado, sendo esta cor determinada pela quantidade de pigmentos externos da semente (Figura 2). A quantidade de pigmentos é determinada por fatores genéticos e ambientais, podendo ser facilmente modificada através de técnicas simples de cultivo (SILVA, 2014). A linhaça dourada desenvolve-se em climas muito frios, como no Canadá e no norte dos Estados Unidos, e a linhaça marrom pode desenvolver-se em regiões

de clima quente e úmido, como no Brasil (MOLENA-FERNANDES et al., 2010). Até 2005, o Brasil produzia somente a variedade marrom, mas, no final de 2006, ocorreu a primeira colheita, de 100 toneladas, da variedade dourada (REDLICH, 2016).

Figura 2 – Sementes de linhaça dourada e marrom.

Fonte: https://www.deilataylor.com/golden-flaxseed-vs-brown-flaxseed/, acessado em 08/05/2017.

2.2.3 Composição Nutricional

Em virtude da presença de componentes alimentares fisiologicamente ativos que podem proporcionar benefícios à saúde, além da nutrição básica, a linhaça é considerada um alimento funcional (HERCHI et al., 2012). A composição desta semente é caracterizada por um alto teor de proteínas vegetais, fibras solúveis e insolúveis, AGPI e compostos fenólicos (KAJLA et al., 2015).

2.2.3.1 Proteínas vegetais

O conteúdo de proteínas oscila, na semente seca, entre 21 e 26%, se destacando não só pela quantidade, mas pela qualidade, pois a linhaça é rica e equilibrada em três aminoácidos diretamente ligados ao desempenho físico e atlético: valina, leucina e isoleucina. As principais proteínas encontradas são a albumina e a globulina, correspondendo a cerca de 20 a 42% do teor proteico total da linhaça (TRUCOM, 2006). Além da qualidade nutricional, essas proteínas apresentam boa absorção de água e óleo, atividade emulsionante e estabilidade (MONEGO, 2009).

2.2.3.2 Fibras alimentares

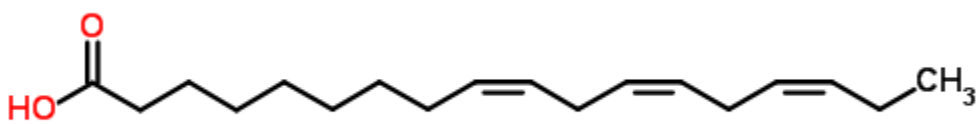
As fibras alimentares presentes na linhaça correspondem a 28% do seu peso seco, sendo que um terço (6 a 11% da semente seca) são fibras solúveis, as quais absorvem a água e se transformam em uma substância gelatinosa que envolve a gordura e impede a sua absorção. Dessa forma, as fibras solúveis contribuem para a melhora da sensibilidade à insulina, redução do colesterol e do risco de DCV. Além disso, as fibras solúveis retardam o tempo de trânsito intestinal e aumentam o volume das evacuações, auxiliando no tratamento da obesidade (TRUCOM, 2006; BLOEDON et al., 2008; REDILICH, 2016).

Já as fibras insolúveis diferem das solúveis por não absorverem água. A função dessas fibras é acelerar o trabalho intestinal e aumentar o bolo fecal, também contribuindo para o tratamento da constipação intestinal e da obesidade. Além disso, as fibras insolúveis são importantes na prevenção do câncer intestinal por protegê-lo do contato com substâncias nocivas (REDLICH, 2016).

2.2.3.3 Óleo de linhaça e ácidos graxos poli-insaturados

A semente de linhaça é uma oleaginosa e, portanto, apresenta elevado teor de lipídios (32 a 38%). O óleo de linhaça é pobre em ácidos graxos saturados (9%), moderado em ácidos graxos monoinsaturados (18%) e rico em ácidos graxos poli-insaturados (73%). Dentre os lipídeos no óleo de linhaça, o ALA, pertencente à família ômega-3, é o ácido graxo principal (39 a 60%) (Figura 3), seguido pelos ácidos oleico (13,4 a 19,4%), linoleico (família ω-6; 12,2 a 17,4%), palmítico (4,9 a 8%) e esteárico (2,2 a 4,6%), o que proporciona uma excelente razão de ácidos graxos ω-6: ω-3 de aproximadamente 0,3:1. Embora o óleo de linhaça seja naturalmente rico em antioxidantes como tocoferóis e betacarotenos, o mesmo se torna facilmente oxidável após ser extraído e purificado. A biodisponibilidade do ALA depende do tipo de linhaça ingerida, tendo maior biodisponibilidade no óleo do que nas sementes moídas ou inteiras (GOYAL et al., 2014; COSTA, 2016).

Figura 3: Estrutura do ácido α-linolênico (ALA).



Fonte: http://www.chemspider.com/Chemical-Structure.4444437.html, acessado em 27/11/2017.

Uma das mais importantes funções dos ácidos graxos ômega-3 e ômega-6 está relacionada à sua conversão enzimática em eicosanoides, principalmente em EPA e DHA. Posteriormente, estes eicosanoides são transformados em prostaglandinas e leucotrienos, os quais desempenham diversas atividades no organismo, como a modulação das respostas inflamatória e imunológica e contribuição na agregação plaquetária, no crescimento e na diferenciação celular (MARQUES, 2008; CUPERSMID et al., 2012).

A ingestão de ALA também provoca alterações estruturais e funcionais na membrana fosfolipídica, aumentando a fluidez da membrana celular, permitindo maior mobilidade das proteínas e favorecendo maior troca de sinais de transdução, interação hormônio-receptor e transporte de substratos entre os meios intra e extracelular. Além disso, o ALA também é capaz de reduzir os níveis de triglicerídeos plasmáticos por inibição da secreção hepática de VLDL e por diminuição da atividade de várias enzimas hepáticas responsáveis pela síntese de triglicerídeos (CUPERSMID et al., 2012).

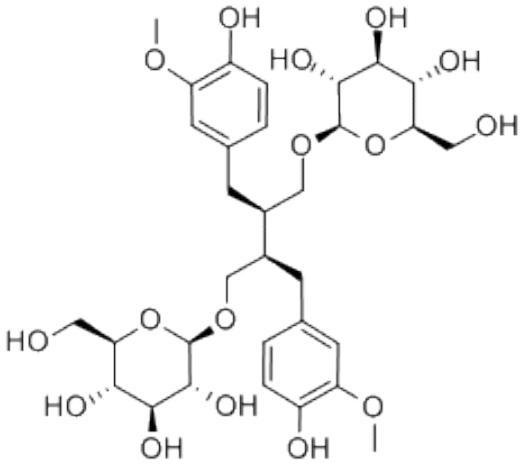
2.2.3.4 Compostos fenólicos

A semente de linhaça também é rica em compostos fenólicos, tais como ácidos fenólicos, flavonoides e lignanas. Os principais ácidos fenólicos presentes na linhaça são os ácidos cafeico, gálico e clorogênico (SCALBERT & WILLIAMSON, 2000), os quais apresentam atividade antisséptica, anticarcinogênica e antioxidante (VIZZOTTO et al., 2010). Os flavonoides são um grupo amplamente distribuído de metabólitos secundários de plantas que apresentam potenciais efeitos antioxidantes, tanto inibindo a deterioração oxidativa de alimentos, quanto provocando efeitos metabólicos benéficos em animais (OOMAH et al., 1996). A linhaça contém de 35-70mg de flavonoides / 100g de semente (TRUCOM, 2006).

Apesar da alta quantidade de ácidos fenólicos e flavonoides, os principais compostos fenólicos presentes na linhaça são as lignanas. As lignanas vegetais são compostos fenólicos formados pela união de dois resíduos de ácido cinâmico e que atuam como antioxidantes e fitoestrógenos. A linhaça contém até 800 vezes mais lignanas do que outros alimentos vegetais, sendo seu teor composto principalmente por SDG (294-700mg / 100g) (Figura 4), matairesinol (0,55mg / 100g), lariciresinol (3,04mg / 100g) e pinoresinol (3,32mg / 100g) (GOYAL et al., 2014). Os benefícios para a saúde das lignanas estão na sua capacidade antioxidante, como sequestradores de radicais hidroxila, e como compostos estrogênicos, devido à sua semelhança estrutural com o 17-β-estradiol. A capacidade antioxidante da SDG está relacionada à supressão das condições oxidantes provocada pelas espécies reativas de oxigênio (ERO). O SDG e sua aglicona, secoisolariciresinol, exibem uma capacidade

antioxidante muito alta e atuam como protetores contra danos ao DNA e aos lipossomas durante o metabolismo das bactérias do cólon que as transformam nas lignanas mamíferas ED e EL (ADLERCREUTZ, 2007; RUBILAR et al., 2010). Além de sua atividade antioxidante e estrogênica, estudos têm demonstrado que o SDG apresenta efeito antitumoral, antiviral, bactericida, inseticida e fungistático, além de proteger contra doenças coronarianas (SMEDS et al., 2007; GOYAL et al., 2014).

Figura 4: Estrutura do secoisolariciresinol diglicosídeo (SDG).



Fonte: http://www.chemicalbook.com/ChemicalProductProperty_EN_CB3506992.htm, acessado em 27/11/2017

2.2.4 Atividades biológicas já descritas

Nos últimos anos, a semente de linhaça e seus componentes isolados têm sido amplamente estudados com o objetivo de elucidar suas principais atividades biológicas e os mecanismos envolvidos. A fim de avaliar o efeito antioxidante da semente de linhaça, Rajesha e colaboradores, em 2006, avaliaram o efeito do pré-tratamento com 0,75 e 1,5g de linhaça / kg, durante 14 dias, sobre a toxicidade do tetracloreto de carbono (CCl_4) em ratos. A linhaça foi capaz de prevenir a redução na atividade das enzimas antioxidantes provocada pela toxina.

Em 2008, Zhang e colaboradores demonstraram que o tratamento com 600mg / dia de SDG, durante 8 semanas, reduziu os níveis de colesterol total e LDL e a glicemia de jejum em indivíduos hipercolesterolêmicos. Este estudo também demonstrou um aumento nos níveis de SECO, ED e EL, constatando uma correlação negativa entre esses metabólitos e os níveis de colesterol plasmático.

Em 2010, Molena-Fernandes e colaboradores avaliaram o efeito das farinhas de linhaça marrom e dourada sobre o perfil lipídico e a evolução ponderal em ratos Wistar,

demonstrando que ambas as variedades promoveram redução significativa nos níveis de triglicerídeos séricos e na razão colesterol total / colesterol HDL após 35 dias de suplementação. No mesmo ano, Moniem e colaboradores avaliaram o efeito do óleo de linhaça na proteção contra o estresse oxidativo induzido por chumbo em ratos, demonstrando proteção contra a peroxidação lipídica e o dano ao DNA, além de promover aumento nos níveis de glutationa reduzida (GSH) e enzimas antioxidantes, como catalase (CAT), superóxido dismutase (SOD), glutationa redutase (GR), glutationa-S-transferase (GST) e glutationa peroxidase (GPx).

Em 2011, Couto e Wichmann demonstraram que a suplementação com 20g de linhaça triturada durante 60 dias foi eficaz na redução do índice de massa corporal (IMC) e da CA de mulheres com IMC superior a 25mg/kg², além de reduzir os níveis de colesterol total, colesterol LDL e triglicerídeos. Ainda em 2011, Abdel-Moneim e colaboradores demonstraram que o óleo de linhaça foi eficiente na proteção contra o dano renal induzido por acetato de chumbo em ratos. Tal proteção foi demonstrada pela melhora na estrutura histológica do rim, assim como pela redução nos níveis de creatinina, uréia, ácido úrico, peroxidação lipídica e produção de óxido nítrico, com concomitante elevação na atividade das enzimas antioxidantes CAT, SOD, GR, GST e GPx. No mesmo ano, Rhee & Brunt demonstraram que a suplementação com 40g diárias de semente de linhaça, durante 12 semanas, reduziu a peroxidação lipídica e a resistência à insulina de indivíduos obesos intolerantes à glicose.

Um estudo realizado por Poudyal e colaboradores, realizado em 2013, avaliou o efeito da suplementação, durante 8 semanas, com ALA, EPA e DHA em um modelo de SM em ratos. Este estudo demonstrou respostas fisiológicas diferentes destes compostos na melhora dos fatores de risco para a SM. O ALA induziu a redistribuição lipídica para longe da área abdominal, melhorou a tolerância à glicose e a sensibilidade à insulina e atenuou a dislipidemia, hipertensão e dimensões, contratilidade, volumes e rigidez do ventrículo esquerdo. O EPA e o DHA aumentaram a ativação simpática e reduziram a adiposidade abdominal e, portanto, a gordura corporal total e atenuaram a resistência à insulina, a dislipidemia, a hipertensão e a rigidez do ventrículo esquerdo. Além disso, os três compostos reduziram a fibrose cardíaca, a esteatose hepática e a inflamação tanto no coração como no fígado.

Egert e colaboradores demonstraram, em 2014, que uma intervenção dietética rica em ALA (3-4g / dia) por 6 meses é eficiente na melhora da função vascular e da inflamação em pacientes com SM, e sugeriram que este efeito estaria relacionado à perda de peso. Outro

estudo, realizado em 2014 por Pilar e colaboradores, demonstrou que a suplementação com 40g / dia de semente de linhaça dourada durante 28 dias foi capaz de melhorar o perfil lipídico e glicêmico, promover proteção hepática, renal e cardíaca, além de reduzir o dano oxidativo a lipídios e proteínas e aumentar as defesas antioxidantes enzimáticas e não-enzimáticas em pacientes com SM.

Em 2015, um estudo de Zuravski e colaboradores avaliou o efeito da suplementação com 40g / dia de semente de linhaça dourada, durante 14 dias, sobre parâmetros de estresse oxidativo em indivíduos saudáveis. Os resultados deste estudo demontraram uma redução do dano oxidativo a lipídios e proteínas, além de uma melhora nas defesas antioxidantes.

2.3 Síndrome Metabólica

2.3.1 Origem

A síndrome metabólica (SM), também conhecida como síndrome da resistência à insulina, síndrome X, síndrome plurimetabólica ou quarteto mortal, corresponde a um distúrbio metabólico complexo conhecido há quase 100 anos (PORTELA, 2010). Este distúrbio foi descrito pela primeira vez em 1923 quando Kylin, um cientista alemão, observou pacientes com o agrupamento de hipertensão, hiperglicemia e gota (KYLIN, 1923). Em 1947, Vague destacou a adiposidade corporal superior como o fenótipo de obesidade que era comumente associada a alterações metabólicas associadas com DM2 e DCV (VAGUE, 1947).

Quatro décadas depois, em 1988, Reaven descreveu este distúrbio de forma mais precisa, reconhecendo-o como o agrupamento de dislipidemia, hipertensão e hiperglicemia, e destacando a resistência à insulina como o principal mecanismo etiológico, sendo estas outras alterações secundárias a esta anormalidade. A partir daí esta síndrome recebeu a denominação de síndrome X e foi reconhecida como um fator de risco para DCV (REAVEN, 1988).

2.3.2 Critérios para o diagnóstico

Embora o conceito de SM tenha sido aceito há bastante tempo, até o ano 2000 nenhuma instituição teve a iniciativa de desenvolver uma definição reconhecida internacionalmente. Na tentativa de chegar a algum acordo sobre a definição, e de fornecer uma ferramenta para clínicos e pesquisadores, uma consulta da Organização Mundial da

Saúde (OMS) propôs um conjunto de critérios. Subsequentemente, o *National Cholesterol Education Program Adult Treatment Panel III* (NCEP-ATP III) e a Federação Internacional de Diabetes (FID) também formularam definições. Essas definições concordam com os componentes essenciais (intolerância à glicose, obesidade, hipertensão e dislipidemia), mas diferem nos detalhes e critérios (ECKEL et al., 2005).

A primeira definição, criada pela OMS em 2000, considerou a resistência à insulina e a intolerância à glicose como componentes essenciais, juntamente com, pelo menos, outros dois fatores: aumento da pressão arterial, hipertrigliceridemia e/ou HDL reduzido, obesidade (medida pela relação cintura-quadril e IMC) e microalbuminúria (WHO, 2000). No entanto, o que dificulta a utilização desta definição é o fato de considerar como ponto de partida a avaliação da resistência à insulina e da intolerância à glicose. Então, à medida que mais informações se tornaram disponíveis esta definição foi sendo substituída.

Em 2001, o NCEP-ATP III desenvolveu uma definição que não exigia a comprovação de resistência à insulina, facilitando a sua utilização. Esta definição teve o foco no risco de DCV e o seu objetivo específico foi facilitar o diagnóstico clínico de indivíduos de alto risco. Segundo o NCEP-ATP III, o diagnóstico da SM requer a presença de três dos cinco componentes: obesidade central, hipertensão arterial, triglicerídeos elevados, colesterol HDL reduzido e hiperglicemia de jejum (Tabela 1). Além de ser mais simples, segundo Lorenzo e colaboradores (2003) a definição do NCEP detecta maior número de indivíduos em risco de DM2 que a definição da OMS.

Tabela 1 - Componentes da Síndrome Metabólica segundo o NCEP-ATP III.

Componentes	**Níveis**
Obesidade abdominal por meio da CA Homens Mulheres	 > 102cm > 88cm
Triglicerídeos	≥ 150 mg/Dl
HDL colesterol Homens Mulheres	 < 40 mg/dL < 50 mg/dL
Pressão arterial	≥ 130mmHg ou ≥ 85mmHg
Glicemia de jejum	≥ 110 mg/dL

Adaptado de I-DBSM, 2005.

No entanto, o principal problema das definições da OMS e do NCEP-ATP III é a aplicabilidade das mesmas nos diferentes grupos étnicos, principalmente relacionado aos pontos de corte para a obesidade, ao quais podem ser diferentes entre as populações de diferentes regiões. Com o objetivo de tentar estabelecer uma definição unificada para a SM e para destacar as áreas onde são necessárias mais investigações sobre a síndrome, um grupo de especialistas foi convocado pela FID, em 2004, para elaborar uma nova definição, a qual tinha o valor limite para a circunferência da cintura como obrigatório, além de pontos de corte específicos para regiões ou países (ALBERTI et al., 2005; ECKEL et al., 2010).

No Brasil, a I Diretriz Brasileira de Diagnóstico e Tratamento da Síndrome Metabólica (I-DBSM) recomenda o uso da definição NCEP-ATP III devido à sua simplicidade e praticidade. Segundo essa diretriz, deve ser realizada investigação clínica e laboratorial com o objetivo de confirmar o diagnóstico da SM (pelos critérios do NCEP-ATP III) e identificar fatores de risco cardiovascular associados (I-DBSM, 2005).

2.3.3 Fisiopatologia

Apesar dos avanços na fisiopatologia e na delineação dos fatores de risco que predispõem à SM, há muitos aspectos-chave que permanecem obscuros. A grande variação na suscetibilidade e idade de início em indivíduos com perfil de risco muito similar sugere uma grande interação entre fatores genéticos e ambientais. Embora a obesidade e a resistência à insulina permaneçam no cerne da fisiopatologia da SM, vários outros fatores como a dislipidemia aterogênica, a suscetibilidade genética, a hipertensão arterial, o estado hipercoagulável, o aumento no estresse oxidativo celular e a atividade do sistema renina-angiotensina-aldosterona podem também estar envolvidos na sua patogênese (KASSI et al., 2011; KAUR, 2014).

Considera-se a obesidade central como um fator fundamental na patogênese da SM, uma vez que o tecido adiposo visceral secreta uma variedade de substâncias bioativas denominadas adipocitocinas, tais como leptina, resistina, fator de necrose tumoral-α (TNF-α), interleucina 6 (IL-6), angiotensina II e inibidor do ativador do plasminogênio 1 (PAI-1) (KASSI et al., 2011). As adipocitocinas integram os sinais endócrinos para mediar múltiplos processos, incluindo sensibilidade à insulina, estresse oxidativo, metabolismo energético, coagulação sanguínea e respostas inflamatórias, as quais aceleram a aterosclerose e a

aterotrombose (KAUR, 2014). Além disso, a adiponectina, uma adipocitocina importante que protege contra o desenvolvimento de DM2, hipertensão, inflamação e doenças vasculares ateroscleróticas, está diminuída em indivíduos com acúmulo de gordura visceral, o que pode estar causalmente relacionado à SM (KASSI et al., 2011).

Outros compostos produzidos pelo tecido adiposo, possivelmente implicados na patogênese da SM, são os ácidos graxos livres (AGL). Na presença de resistência à insulina, o processo de mobilização dos AGL a partir dos triglicerídeos do tecido adiposo é acelerado. No fígado, os AGL resultam (devido à resistência insulínica hepática) no aumento da produção de glicose e triglicerídeos e secreção de VLDL, mantendo um ciclo vicioso (KASSI et al., 2011).

Além disso, a hipertensão arterial é frequentemente associada a várias anormalidades metabólicas, das quais a obesidade, a intolerância à glicose e a dislipidemia são as mais comuns. Estudos sugerem que tanto a hiperglicemia como a hiperinsulinemia ativam o sistema renina-angiotensina-aldosterona, aumentando a expressão do angiotensinogênio, da angiotensina II e do receptor AT1, o que, em conjunto, pode contribuir para o desenvolvimento de hipertensão em pacientes com resistência à insulina. Há também evidências de que a resistência à insulina e a hiperinsulinemia levam à ativação do sistema nervoso simpático e, como resultado, os rins aumentam a reabsorção de sódio, o coração aumenta o débito cardíaco e as artérias respondem com vasoconstrição, resultando em hipertensão arterial (KAUR, 2014).

A SM também está associada a um aumento do estresse oxidativo celular, devido à superprodução de ERO, as quais podem induzir resistência à insulina, que é indispensável para a progressão deste distúrbio. Além disso, as ERO ativam receptores mineralocorticóides e o sistema nervoso simpático, o que pode contribuir ainda mais para a progressão da SM (ANDO & FUJITA, 2009).

2.3.4 Prevenção e Tratamento

A identificação e o manejo clínico dos pacientes com SM são importantes para implementar adequadamente os tratamentos e, assim, reduzir o risco de doenças subsequentes. Abordagens preventivas eficazes incluem mudanças de estilo de vida, principalmente perda de peso, com dieta e exercício, e o tratamento compreende o uso adequado de agentes farmacológicos e/ou cirúrgicos para reduzir os fatores de risco específicos (KAUR, 2014).

O NCEP-ATP III recomenda que a obesidade seja o principal alvo de intervenção para a SM (NCEP-ATP III, 2001). A terapia de primeira linha deve ser a redução de peso, através de uma alimentação saudável e com o aumento da atividade física. A perda de peso reduz o colesterol e os triglicerídeos séricos, aumenta o colesterol HDL, reduz a pressão arterial, a glicemia e a resistência à insulina (GRUNDY et al., 2004).

A alimentação adequada para o tratamento da SM deve incluir uma dieta hipocalórica, com redução da ingestão de gorduras, substituindo o consumo de gorduras saturadas por insaturadas e reduzindo o consumo de gorduras trans (hidrogenadas), aumento da ingestão de frutas, hortaliças, leguminosas e cereais integrais e redução da ingestão de açúcares livres e sódio. Já a atividade física é determinante no gasto de calorias e fundamental para o balanço energético e controle do peso. A atividade física regular diminui o risco relacionado a cada componente da SM, uma vez que reduz a pressão arterial, eleva o colesterol HDL e melhora o controle glicêmico. O exercício físico deve ter duração mínima de 30 minutos, preferencialmente diário, incluindo exercícios aeróbicos e de fortalecimento muscular (I-DBSM, 2005).

O tratamento farmacológico deve ser considerado para aqueles pacientes cujos fatores de risco não são adequadamente reduzidos com as mudanças de estilo de vida. A gestão clínica da SM é difícil porque não existe um método reconhecido para prevenir ou melhorar a síndrome como um todo. Assim, a recomendação é tratar cada componente separadamente, colocando uma ênfase particular sobre os componentes que são facilmente passíveis de tratamento medicamentoso (KAUR, 2014).

A obesidade pode ser tratada com medicamentos como dietilpropiona, femproporex, mazindol, sibutramina e orlistat para pacientes com obesidade (IMC $\geq$ 30 kg/m²) ou excesso de peso (IMC entre 25 e 30 kg/m²). Para os pacientes com obesidade mórbida (IMC > 40 kg/m²) é recomendada, sob algumas condições, a cirurgia bariátrica (I-DBSM, 2005). O tratamento da dislipidemia aterogênica pode ser realizado com o uso de estatinas ou fibratos, sendo ambos capazes de reduzir o risco de DCV. Já a hipertensão arterial deve ser controlada com o uso dos medicamentos anti-hipertensivos recomendados pela VI Diretrizes Brasileiras de Hipertensão, tais como diuréticos, betabloqueadores, antagonistas de cálcio, inibidores da enzima conversora de angiotensina e bloqueadores dos receptores de angiotensina II, sendo que o recomendado é a utilização da associação de fármacos (VI-DBH, 2010). Quando o DM2 está presente deve ser feita terapia com hipoglicemiantes, sendo recomendadas as combinações terapêuticas de metformina e glitazonas, metformina e sulfoniluréias, e glitazonas e sulfoniluréias (I-DBSM, 2005).

Além das mudanças no estilo de vida e dos tratamentos medicamentoso e cirúrgico, alguns estudos demonstraram efeitos benéficos de alimentos funcionais como adjuvantes no tratamento e na prevenção da SM (WU et al., 2010; BLAND, 2011; KHAN et al., 2013; PILAR et al, 2014). De acordo com Bland (2011), os principais componentes responsáveis por esses efeitos benéficos são os compostos fitoquímicos presentes nestes alimentos. Especificamente, alguns compostos podem diferencialmente modular o perfil da expressão gênica pós-prandial em pessoas com SM e ajudar a normalizar as funções metabólicas perturbadas associadas a esta condição. Além disso, estudos têm demonstrado que a ingestão dietética de ácidos graxos ômega-3 presentes em muitos destes alimentos também diminui o risco de doenças cardíacas. Os dados obtidos a partir de vários ensaios indicam que a suplementação dietética com esses ácidos graxos reduz significativamente o risco de síndromes como doenças cardíacas coronárias e insuficiência cardíaca súbita, principalmente pela capacidade dos mesmos em reduzir a hipertrigliceridemia (I-DBSM, 2005; KHAN et al., 2013). Por fim, a presença de compostos com atividade antioxidante nestes alimentos funcionais tem demonstrado ter relação direta com a redução dos fatores de risco associados à SM, visto que o estresse oxidativo é um fator de destaque na fisiopatologia deste distúrbio (SANTOS et al., 2006).

2.3.5 Modelos animais

A ocorrência disseminada de SM em humanos indica a necessidade urgente de estudar as causas e a progressão dos sintomas desse distúrbio. Estes estudos requerem modelos animais viáveis que imitem adequadamente todos os aspectos da SM humana, especialmente o desenvolvimento de obesidade, hiperglicemia, dislipidemia, hipertensão, doença hepática gordurosa e disfunção renal (PANCHAL & BROWN, 2011).

Ratos e camundongos têm sido utilizados há muitos anos como modelos de doenças humanas, visando a investigação da progressão dos sintomas dessas doenças. Vários modelos já foram descritos para o estudo da SM, entre eles é possível destacar a utilização de dietas ricas em carboidratos, como a frutose e a sacarose, ou aquelas que incluam uma quantidade significativa de gorduras de origem animal ou de óleos vegetais (IYER et al., 2009).

No entanto, a utilização de frutose é o modelo mais bem estabelecido para a indução de SM em roedores, uma vez que é o modelo que modula de forma mais fidedigna a doença humana (CAMPOS & TAPPY, 2016; PANCHAL & BROWN, 2011). Já foi demonstrado que

uma dieta rica em frutose é capaz de provocar hipertensão, resistência à insulina, hipertrigliceridemia, aumento da produção hepática de VLDL e hiperglicemia (NAKAGAWA et al., 2006; BARROS et al., 2007; RONCAL et al., 2009), apesar de haverem controvérsias quanto à capacidade da mesma em provocar obesidade (PATEL et al., 2009; BOCARSLY et al., 2010).

Uma das razões pelas quais a frutose provoca SM é porque ela não é capaz de estimular a secreção de insulina das células β-pancreáticas, o que ocorre devido à ausência de transportador GLUT5 nestas células. Além disso, o metabolismo da frutose ignora a principal via da glicólise que converte a frutose-6-fosfato em frutose-1,6-bifosfato pela enzima fosfofrutoquinase. Estes dois fatores contrariam o metabolismo da glicose, o qual estimula a secreção de insulina a partir da célula β-pancreática, promovendo a conversão de glicose em glicogênio (MAMIKUTTY et al., 2014).

Quanto à forma de administração da frutose em animais experimentais, estudos já relataram que a adição de 60% de frutose na ração padrão ou de 10% a 20% na água de beber são eficientes para a indução de SM (LEIBOWITZ et al., 2013; GUIMARAES et al., 2014).

3 OBJETIVOS

3.1 Objetivo Geral

Avaliar os efeitos do óleo de linhaça (OL) e da lignana da linhaça secoisolariciresinol diglicosídeo em um modelo de síndrome metabólica em ratos Wistar.

3.2 Objetivos específicos

- Avaliar a eficácia do tratamento com solução de frutose a 30% na indução de SM em ratos Wistar;
- Analisar o efeito da suplementação com OL ou SDG durante este período na prevenção do desenvolvimento da SM;
- Determinar o efeito da solução de frutose e/ou da suplementação com OL ou SDG sobre marcadores inflamatórios;
- Determinar o efeito da solução de frutose e/ou da suplementação com OL ou SDG sobre a estrutura histopatológica e os marcadores sanguíneos de função renal e hepática.
- Avaliar a associação da SM com alterações em parâmetros oxidativos;
- Avaliar o efeito do OL e/ou do SDG na proteção contra o estresse oxidativo associado à SM.

PARTE II

4 ARTIGO

Protective Role of Flaxseed Oil and Flaxseed Lignan Secoisolariciresinol Diglucoside against Oxidative Stress in Rats with Metabolic Syndrome

Publicado no periódico "Journal of Food Science: Health, Nutrition, and Food"

Protective Role of Flaxseed Oil and Flaxseed Lignan Secoisolariciresinol Diglucoside Against Oxidative Stress in Rats with Metabolic Syndrome

Bruna Pilar, Angélica Güllich, Patrícia Oliveira, Deise Ströher, Jacqueline Piccoli, and Vanusa Manfredini

Abstract: This study evaluated the protective effect of flaxseed oil (FO) and flaxseed lignan secoisolariciresinol diglucoside (SDG) against oxidative stress in rats with metabolic syndrome (MS). 48 rats were allocated into the following 6 groups: Groups 1 (control), 5 (FO), and 6 (SDG) received water and were treated daily orally with saline, FO, and SDG, respectively. Groups 2 (MS), 3 (MS+FO), and 4 (MS+SDG) received 30% fructose in drinking water for MS induction and were treated daily orally with saline, FO, and SDG, respectively. After 30 d, animals were sacrificed, and blood was collected for biochemical and oxidative analysis. Body weight was recorded weekly. Systolic blood pressure (SBP) was measured before and after treatment. Fructose could produce MS and oxidative stress. FO and SDG prevented changes in SBP, lipids, and glucose. FO and SDG prevented oxidative damage to lipids, and only FO prevented oxidative damage to proteins associated to MS. FO and SDG improved enzymatic antioxidants defenses and reduced glutathione levels, which was greater with SDG. Total polyphenol levels were enhanced in groups that received SDG. Thus, the results of this study demonstrated that treatment with a 30% fructose solution for 30 d is effective for MS induction and the oxidative stress is involved in the pathophysiology of MS induced by fructose-rich diets. Furthermore, we demonstrated that the antioxidant effects attributed to flaxseed are mainly due to its high lignan content especially that of SDG, suggesting that this compound can be used in isolation to prevent oxidative stress associated with MS.

Keywords: antioxidant, flaxseed oil, metabolic syndrome, oxidative stress, secoisolariciresinol diglucoside

Practical Application: We report that the antioxidant effects attributed to flaxseed are mainly due to its high lignan content, especially that of secoisolariciresinol diglucoside. This is significant because suggests that this compound can be used in isolation to prevent oxidative stress associated with MS. Furthermore, this study was the only one to perform a comparison of the abilities of 2 components of flaxseed to protect against oxidative stress in an MS model, which brings a great advance in the medicine's field, since it indicates another alternative for improve the health and the quality of life of patients with this disorder.

Introduction

The term metabolic syndrome (MS) has been used by researchers to define a set of risk factors of metabolic origin, which has a high socioeconomic cost and is considered a worldwide epidemic. According to a more recent definition proposed by the International Diabetes Federation, MS corresponds to a combination of at least 3 of the following risk factors: visceral obesity, hypertension, increased triglyceride and glucose levels, and decreased high density lipoprotein (HDL) cholesterol levels (Kassi and others 2011).

Furthermore, oxidative stress plays a central role in MS pathology and can be a key factor in its progression. Reactive oxygen species (ROS) are highly reactive derivatives of oxygen metabolism. Under physiological conditions, a balance is maintained between ROS production and their removal by enzymatic and nonenzymatic antioxidants. Under pathological states, such as in MS, an increase in oxidative capacity along with a decrease in antioxidant capacity creates an imbalance that results in oxidative stress, which has toxic effects on cells and tissues (Hutcheson and Rocic 2012).

The high prevalence of MS and its associated health consequences demonstrates the need to define strategies to prevent it, and many studies indicate that increased intake of functional foods and antioxidant supplements is inversely related to the presence of risk factors associated with MS (Andresen and Fernandez 2013; Pattyn and others 2013).

Flaxseed (*Linum usitatissimum* L.) is a functional food that has attracted growing interest from researchers because it contains biologically active components such as dietary fibers, plant proteins, polyunsaturated fatty acids (PUFAs), and lignans, which play a beneficial role in the organism, enabling disease prevention (Rubilar and others 2010; Taylor and others 2010; Kajla and others 2015). Flaxseed oil (FO) contains approximately 57% alpha-linolenic acid (ALA), being the richest ALA source. It has been shown in previous studies that eicosapentaenoic acid and docosahexaenoic acid, which are formed from ALA metabolism, reduce this risk of chronic diseases, such as atherosclerosis, cardiovascular disease, cancer, and hyperlipidemia (Rodriguez-Leyva and others 2010). In addition, flaxseed is rich in lignans, phytoestrogens commonly consumed in the human diet, and secoisolariciresinol

JFDS-2017-1207 Submitted 7/24/2017, Accepted 10/2/2017. *Authors Pilar, Güllich, Oliveira, Ströher, and Manfredini are with Postgraduate Program in Biochemistry, Federal Univ. of Pampa, Uruguaiana, Rio Grande do Sul, Brazil. Author Piccoli is with Postgraduate Program in Pharmaceutical Sciences, Federal Univ. of Pampa, Uruguaiana, Rio Grande do Sul, Brazil. Direct inquiries to author Pilar (E-mail: brunapilar16@hotmail.com).*

doi: 10.1111/1750-3841.13964

Journal of Food Science A Publication of the Institute of Food Technologists

diglucoside (SDG) is the main lignan found (approximately 1% by dry weight) (Khalesi and others 2011). After its metabolism in the intestine and colon, SDG is broken down into secoisolariciesinol, enterodiol (ED), and enterolactone (EL) molecules, which have already shown health beneficial effects (Hu and others 2007; Zhang and others 2008).

Furthermore, studies have shown that flaxseed can reduce oxidative damage, including in MS patients (Pilar and others 2014; Zuravski and others 2015). However, some studies attribute these effects to FO and its high ALA concentration (Karaka and Eraslan 2013; Badawy and others 2015), whereas other studies indicate that the antioxidant effects of flaxseed are due to the presence of its lignans, especially SDG (Hu and others 2007; Newary and Abdou 2009). Thus, to clarify the component responsible for the antioxidant effects attributed to flaxseed in MS, the main objective of this study was to evaluate the comparative effects of FO and flaxseed lignan SDG on oxidative parameters in rats with this disorder.

Materials and Methods

Chemicals

All the chemicals used were of analytical grade. All reagents were obtained from Sigma Chemical Co. (St. Louis, Mo., U.S.A.).

FO and sDG

FO was obtained from Mundo dos Óleos Company (Brasília, DF, Brazil) in July 2015. According to the manufacturer's information, it was obtained by cold pressing and filtration from seeds. SDG was obtained from Sigma Chemical Co., with a purity ≥97% (high performance liquid chromatography). A solution of 5 mg/mL SDG was prepared for administration in the rats.

Ethical standards

All experiments were approved by the Ethics Committee on Animal Use of the Federal Univ. of Pampa, Uruguaiana, Rio Grande do Sul, Brazil (Protocol 024/2014).

Gas chromatography-mass spectrometry analysis of FO

About 1 μL of an aliquot of FO was subjected to gas chromatography–mass spectrometry (GC-MS) analysis that was performed using a Shimadzu model GC/MS QP-2010Plus (Shimadzu Corporation, Kyoto, Japan). GC was equipped with an RTX-5MS capillary column (30 m × 0.25 mm i.d. × 0.25 μm film thickness) consisting of a stationary phase of 5% diphenyl and 95% dimethyl polysiloxane. The injector temperature was 250 °C, and the split vent was closed for a minute (splitless operation). Helium gas was used as a carrier gas with a flow rate of 1.0 mL/min. The oven temperature programming was as follows: the initial oven temperature was maintained at 50 °C for 5 min and then increased to 300 °C at a rate of 10 °C/min maintained for 30 min. The temperature of the ion source and the transfer line was 290 °C. Identification of the compounds was performed by comparing their mass spectra with NIST library available in the instrument.

Experimental animals and diets

Forty-eight male Wistar rats, 30 d old, were lawfully acquired from the Central Animal Laboratory of Univ. Federal of Santa Maria, Rio Grande do Sul, Brazil, and acclimated for 30 d with standard chow and water *ad libitum*. Animals were housed in cages under standard conditions (22 ± 1 °C, 55 ± 5% humidity, light-dark cycle 12/12 h).

Rats were randomly divided into 6 groups ($n = 8$). Groups 2 to 4 received a 30% fructose solution in drinking water during the experimental period (30 d) for MS induction, while groups 1, 5, and 6 received water *ad libitum*. Simultaneously, during these 30 d, the animals received daily treatment by oral gavage, as described below (Penumathsa and others 2008, Kaithwas and Majumdar 2012):

Group 1(control): 1 mL/kg body weight saline;
Group 2 (MS): 1 mL/kg body weight saline;
Group 3 (MS+FO): 1 mL/kg body weight FO;
Group 4 (MS+SDG): 20 mg/kg body weight SDG;
Group 5 (FO): 1 mL/kg body weight FO;
Group 6 (SDG): 20 mg/kg body weight SDG;

Twenty-four hours after the last administration, animals were anesthetized and euthanized by decapitation and whole blood was collected.

Characterization of MS

For characterization of MS, body weight; blood pressure; and glucose, triglyceride, and HDL cholesterol levels were evaluated. Body weight was recorded weekly. Systolic blood pressure (SBP) was measured by noninvasive tail-cuff plethysmography (AD Instruments Pty Ltd, Bella Vista, NSW, Australia) before and after supplementation in conscious rats. Before measurement, the rats were kept at 30 °C for 10 min, in an acrylic containment apparatus (Insight - Equipment, Research and Teaching, Ribeirão Preto, SP, Brazil) to make the pulsations of the tail artery detectable. To establish the SBP value, 10 measurements were taken, and the average was obtained. To minimize stress-induced variations in blood pressure, all measurements were taken by the same person in the same peaceful environment. Moreover, to guarantee the reliability of the measurements, we established a training period of 1 wk before the actual trial time, and during this period, the rats were accustomed to the procedure. Glucose, triglyceride, and HDL cholesterol measurements were carried out in triplicate using the A25 Biosystems automatic analyzer (Barcelona, Spain) for *in vitro* diagnostics

Oxidative damage

For evaluation of oxidative damage to lipids, proteins and DNA, lipid peroxidation, and protein carbonyls were determined in plasma, and the micronucleus rate and comet assay were evaluated in whole blood leukocytes. Lipid peroxidation was performed using a thiobarbituric acid-reactive substances (TBARS) assay as described by Ohkawa and others (1979). This method involves the reaction of a degradation product of lipid peroxidation, malondialdehyde (MDA), with TBA under conditions of high temperature and acidity to generate a colored adduct that is measured spectrophotometrically. Protein carbonyls were measured using a spectrophotometric method described by Levine and others (1990), which involves the reaction of plasma samples with 2,4-dinitrophenylhydrazine (DNPH) in 6 M guanidine hydrochloride, which binds selectively to protein carbonyl groups. The micronucleus test was performed according to Schmid (1975). For this analysis, blood smears were produced and stained by hematological staining. After drying, the smears were analyzed under an optical microscope to establish the frequency of micronuclei. The alkaline comet assay was carried out as described by Singh and others (1988). A suspension of leukocyte cells was spread on a glass slide and, after lysis, electrophoresis was performed for 20 min (25V, 300 mA). The slides were stained with silver nitrate

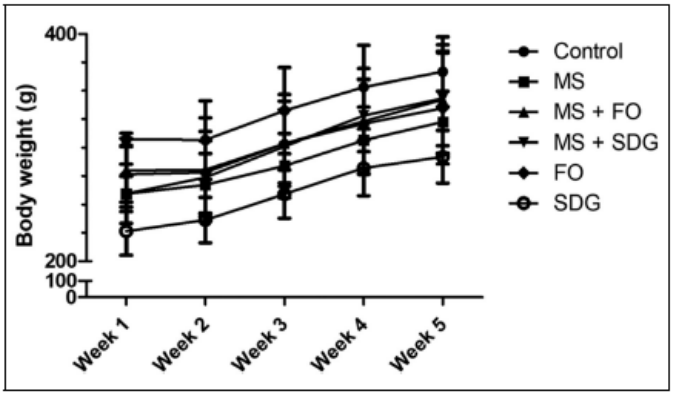


Figure 1–Body weight gain of rats during the experimental period. There was no significant difference in body weight gain between groups. MS, metabolic syndrome; FO, flaxseed oil; SDG, secoisolariciresinol diglucoside.

and analyzed under an optical microscope. Cells were scored visually according to tail length from 0 (no migration) to 4 (maximum migration), and the damage index ranged from 0 (all cells without migration) to 400 (all cells with maximum migration). All assays were carried out in triplicate.

Antioxidant defenses

Nonenzymatic antioxidant defenses were measured by total polyphenol and reduced glutathione (GSH) levels. Plasma samples were analyzed for total polyphenol (TP) content using a modified Folin-Ciocalteu colorimetric method (Singleton and others 1999). TP content was standardized against gallic acid and expressed as gallic acid equivalents (GAE). GSH levels were measured in erythrocytes according Akerboom and Sies (1981). In this method, GSH is measured by reaction with 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB) to form the yellow derivative, 5′-thio-2-nitrobenzoic acid (TNB), which can be measured spectrophotometrically.

In addition, enzymatic antioxidant defenses were evaluated by determining the activity of the enzymes catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx). CAT activity was assayed spectrophotometrically in erythrocyte samples by the method of Aebi (1984), which involves monitoring the disappearance of H_2O_2 in the presence of CAT. SOD activity in erythrocytes was determined using a RANSOD kit (Randox Brazil LTDA, Belo Horizonte, MG, Brazil), which uses xanthine and xanthine oxidase to generate superoxide radicals that react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (I.N.T.) to form a red formazan dye, which is assessed spectrophotometrically. GPx activity in erythrocytes was achieved using the RANSEL kit (Randox LTDA Brazil, Belo Horizonte, MG, Brazil). This kit is based on the reaction of GPx with GSH, which is oxidized to oxidized glutathione (GSSG) and, in the presence of glutathione reductase (GR), GSSG is converted back to GSH at the expenses of NADPH. NADPH decline is then monitored spectrophotometrically. All assays were carried out in triplicate.

Statistical analysis

Data were expressed as mean ± standard deviation (SD). SBP and body weight data were analyzed by two-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparisons test for *post hoc* analysis. For the remaining data, the comparison between groups was performed using one-way ANOVA followed by Bonferroni's post hoc test. Results were considered statistically significant when $P < 0.05$. Statistical analyses were performed using GraphPad Prism software (version 5.0, GraphPad Software, Inc., La Jolla, San Diego, Calif., U.S.A.).

Results

GC-MS analysis of fO

GC-MS analysis of FO identified the following fatty acids: palmitic, C16:0 (1.33%); stearic, C18:0 (0.95%); oleic, C18:1 (5.36%); linoleic, C18:2 (3.9%); and linolenic, C18:3 (13, 8%) acids. In addition, steroids (cholesterol, stigmasterol, sitosterol, fucosterol and lanosterol), tocopherols (α and γ), and other less abundant constituents such as aldehydes, ketones, alcohols, esters, alkanes, and heterocyclic compounds were identified.

Body weight

Figure 1 shows the animal's body weight during the treatment period. There was no significant difference in body weight gain between the groups during the treatment period.

Systolic blood pressure

Treatment with fructose solution for 30 d significantly increased ($P < 0.05$) SBP in the group that received fructose solution (MS). In addition, treatment with FO and SDG could prevent this alteration caused by the fructose solution. In healthy rats, FO and SDG

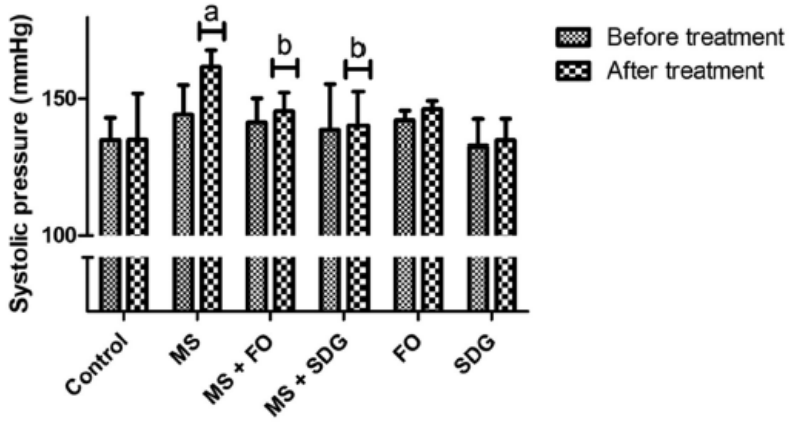


Figure 2–Systolic blood pressure in different study groups before and after 30 d of FO and SDG supplementation. Data are expressed as mean ± SD. (a) $P < 0.05$ compared to control group. (b) $P < 0.05$ compared to MS group. MS, metabolic syndrome; FO, flaxseed oil; SDG, secoisolariciresinol diglucoside. 10 measurements were taken to establish the SBP value.

Table 1–Biochemical parameters in different study groups.

	Glucose (mg/dL)	Triglycerides (mg/dL)	HDL cholesterol (mg/dL)
Control	109.7 ± 20.7	107.7 ± 10.3	48.3 ± 0.4
MS	151.6 ± 14.8[a]	312.8 ± 2.5[a]	24.4 ± 1.9[a]
MS+FO	144.0 ± 10.8[a,c]	172.8 ± 30.3[a,b,c]	41.5 ± 5.2[a,b,c]
MS+SDG	107.8 ± 10.6[b]	196.0 ± 65.7[a,b,d]	31.3 ± 1.6[a,b,d]
FO	115.6 ± 10.6	110.8 ± 14.5	49.3 ± 5.5
SDG	104.6 ± 17.9	108.2 ± 10.9	47.1 ± 1.8

Values expressed as mean ± S.D.
[a] $P < 0.05$ compared to control group.
[b] $P < 0.05$ compared to MS group.
[c] $P < 0.05$ compared to FO group.
[d] $P < 0.05$ compared to SDG group. MS, metabolic syndrome; FO, flaxseed oil; SDG, secoisolariciresinol diglucoside. All experiments were performed in triplicate.

did not cause significant changes in SBP increase when compared to the control group (Figure 2).

Biochemical analysis

Table 1 shows the results for glucose, triglyceride, and HDL cholesterol levels in different groups. Treatment with 30% fructose solution for 30 d significantly increased ($P < 0.05$) glucose and triglycerides levels, and produced a statistically significant reduction ($P < 0.05$) in HDL cholesterol levels compared with the control group. Treatment with FO and SDG prevented the reduction in HDL levels and the increase in triglycerides levels. The increase in glucose levels was prevented just in groups that received SDG, with no significant differences in the group that received FO when compared with the MS group. Furthermore, FO and SDG did not promote statistically significant changes in the healthy rats when compared to the control group.

Oxidative stress parameters

Figure 3 shows the results of oxidative damage biomarkers in different study groups. Figure 3A shows lipid peroxidation assessed by measuring TBARS. TBARS levels were significantly higher ($P < 0.05$) in the MS group when compared with the control group. MS+FO and MS+SDG group showed significantly lower values ($P < 0.05$) than the MS group. Figure 3B shows the carbonyl group levels, where we can observe significantly higher ($P < 0.05$) values in the MS group and in MS+SDG group than in the control group. MS+FO group showed significantly lower carbonyl group levels ($P < 0.05$) than control and MS groups. Moreover, the FO and SDG groups presented levels similar to those of the control group in TBARS and carbonyl group levels. Figure 3C and D show the frequency of micronuclei and results of the comet

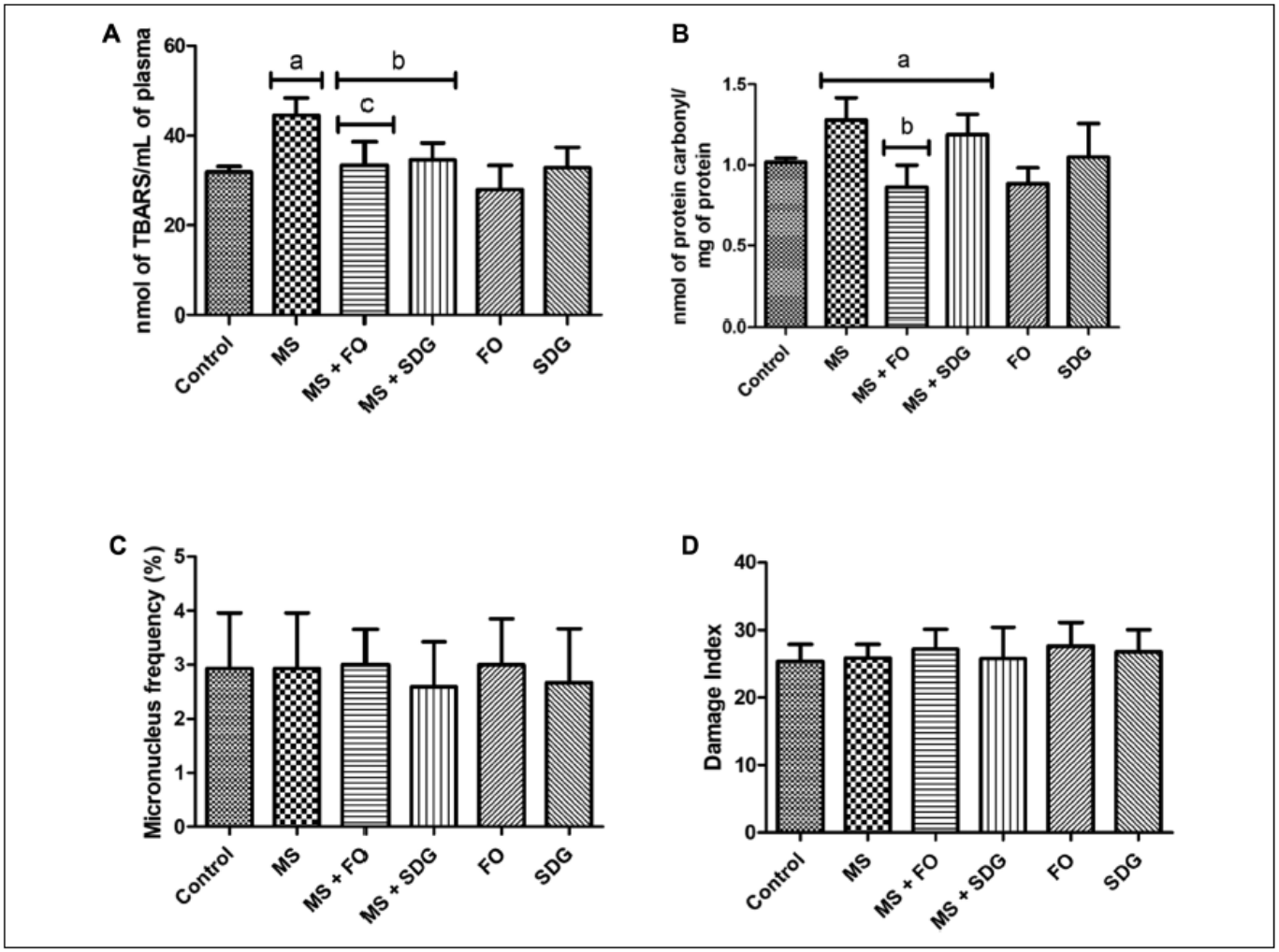


Figure 3–Biomarkers of oxidative damage in different study groups. (A) Lipid peroxidation (TBARS). (B) Protein carbonyl levels. (C) Micronucleus test. (D) Comet assay. Data are expressed as mean ± SD. (a) $P < 0.05$ compared to control group. (b) $P < 0.05$ compared to MS group. (c) $P < 0.05$ compared to FO group. MS, metabolic syndrome; FO, flaxseed oil; SDG, secoisolariciresinol diglucoside. All assays were carried out in triplicate.

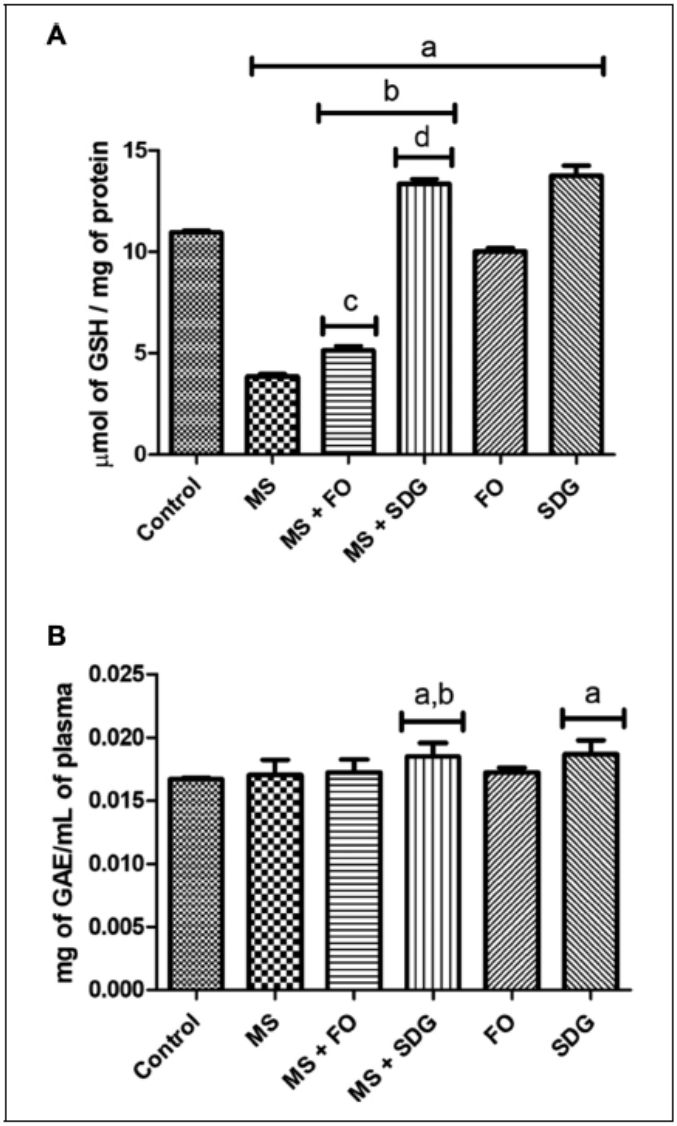


Figure 4–Nonenzymatic antioxidant levels in different study groups. (A) GSH levels. (B) Total polyphenol levels. Data are expressed as means ± SD. (a) $P < 0.05$ compared to control group. (b) $P < 0.05$ compared to MS group. (c) $P < 0.05$ compared to FO group. (d) $P < 0.05$ compared to SDG group. GAE, gallic acid equivalent; MS, metabolic syndrome; FO, flaxseed oil; SDG, secoisolariciresinol diglucoside. All assays were carried out in triplicate.

assay, wherein no significant differences were observed between the groups.

Antioxidant defenses

Figure 4 shows the concentrations of compounds responsible for nonenzymatic antioxidant defenses. GSH levels are expressed in Figure 4A, wherein it was possible to observe a significant reduction ($P < 0.05$) in the group that received the fructose solution (MS). Furthermore, supplementation with FO and SDG significantly prevented ($P < 0.05$) this reduction, and the results were greater in groups that received SDG. Furthermore, FO and SDG increased significantly ($P < 0.05$) GSH levels in healthy rats. Figure 4B shows total polyphenol levels, wherein MS + SDG and SDG groups presented significantly higher values ($P < 0.05$) than the MS and control groups.

Antioxidant enzyme activities are shown in Figure 5. The groups that received FO and SDG presented significantly higher ($P < 0.05$) CAT activity than the control and MS groups (Figure 5A). However, no significant differences were observed ($P < 0.05$) between the control and MS groups. For SOD and GPx, there was a significant reduction ($P < 0.05$) in their activities in the MS group when compared with the control group. FO and SDG prevented this reduction, which was greater in groups that received SDG. In addition, FO and SDG significantly increased ($P < 0.05$) the SOD and GPx activities in healthy rats, also being greater with SDG (Figure 5B and C).

Discussion

In this study, we evaluated the comparative effects of FO, an ALA rich oil, and SDG, a lignan found in high concentration in flaxseed, against oxidative stress in rats with MS. It is known that high fructose consumption is one of the factors responsible for obesity development and other changes observed in MS, and these effects are independent of caloric intake (Roncal and others 2009). Thus, diets with high fructose concentrations (10% to 60%) have been used to induce MS in animal models (Leibowitz and others 2013, Guimaraes and others 2014). The results showed that 30% fructose solution administered for 30 d promoted an increase in SBP and glucose and triglyceride levels and reduce HDL cholesterol levels, demonstrating that this is an effective model for MS induction in Wistar rats. In addition, both FO and SDG partially prevented these changes, in agreement with the findings of previous studies (Ogawa and others 2009; Tzang and others 2009; Park and Velasquez 2012).

Oxidative stress refers to several harmful processes resulting from excessive ROS formation associated with limited antioxidant defenses, playing an important role in the pathogenesis of several diseases (Elnakish and others 2013). These excess ROS attack important biomolecules and cells, leading to lipid, protein, and DNA damage, which were evaluated in this study (Reuter and others 2010). Lipids are essential biological membrane components and represent prime targets for ROS attack. This attack results in lipid peroxidation, which leads to the formation of highly reactive breakdown products, and MDA is the main product (Barrera 2012). In addition, proteins are an important ROS target, because they are abundant in cells, plasma, and most tissues; besides having a high reaction rate (Pandey and others 2010). ROS attack proteins, primarily affecting amino acid side chains, and carbonyl group formation is one of the main consequences of this attack. Detection and quantification of these groups has become the most accepted method for measuring oxidative damage to proteins in situations involving oxidative stress (Tamarit and others 2012). Furthermore, DNA has also been studied as a ROS target, wherein DNA lesions can block genome replication and transcription and, if not remedied, may lead to mutations or aberrations on a large scale, threatening cells viability.

Increases in TBARS and carbonyl group levels observed in this study after treatment with fructose demonstrated that aspects of MS are associated with oxidative damage to lipids and proteins, which agrees with findings from previous studies (Armutcu and others 2008; Park and others 2013). On the other hand, although studies have shown that DNA damage may play an important role in many diseases, no evidence has been found in the literature demonstrating cytogenetic alterations in individuals with MS, which agrees with the results of this study.

In line with previous findings, this study also demonstrated that FO could protect against protein and lipid damage in groups that received fructose solution (MS) (Guermouche and others 2014; Pilar and others 2014). Furthermore, flaxseed lignan SDG

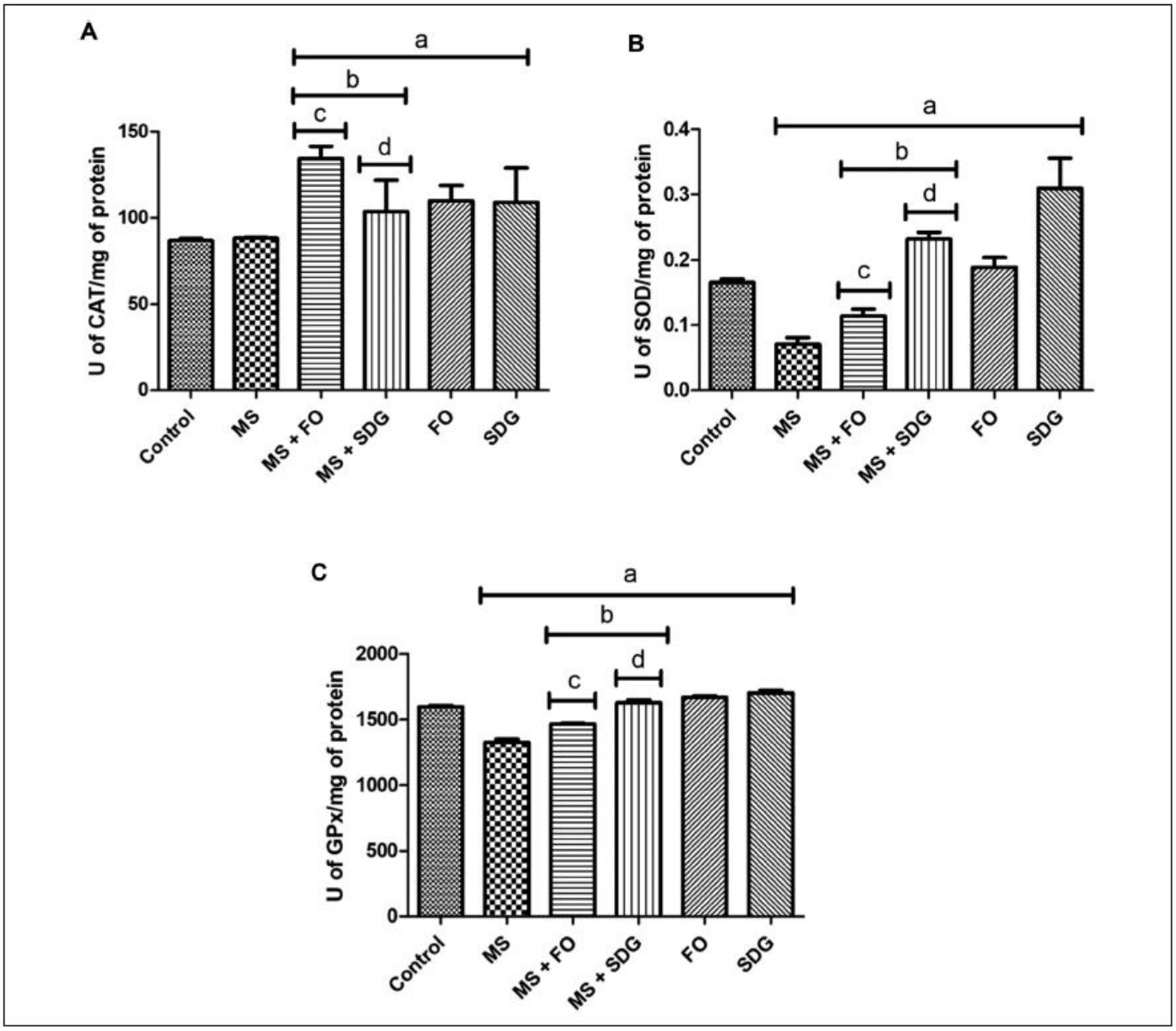


Figure 5–Enzymatic antioxidant defenses in different study groups. (A) CAT activity. (B) SOD activity. (C) GPx activity. Data are expressed as means ± SD. (a) $P < 0.05$ compared to control group. (b) $P < 0.05$ compared to MS group. (c) $P < 0.05$ compared to FO group. (d) $P < 0.05$ compared to SDG group. MS, metabolic syndrome; FO, flaxseed oil; SDG, secoisolariciresinol diglucoside. All assays were carried out in triplicate.

protection against oxidative damage to lipids agrees with results of Newairy and Abdou (2009). They demonstrated that a flax-lignan complex protected against lead acetate-induced lipid peroxidation in rats. However, FO and SDG did not reduce oxidative damage to lipids and proteins in groups that did not receive fructose solution when compared with the control group. This result confirms the involvement of oxidative stress in MS induced by fructose-rich diets, since only in the groups which the MS development was prevented, the protection against oxidative damage was also observed. In the micronucleus frequency and comet assay, all groups exhibited normal values, demonstrating that the components of flaxseed are not able to produce cytogenetic changes. These findings are consistent with previous studies (Pilar and others 2014; Zuravski and others 2015).

Antioxidants play an important role in the body, protecting it against free radical damage, and those obtained through the diet (exogenous) have been widely studied for their numerous disease protective mechanisms (Pérez-Jimenez and others 2015). In this study, no significant differences were found in total polyphenol content between the control and MS groups, demonstrating that, although MS is related to an increase in oxidative damage, it does not interfere with polyphenol content. In the supplemented groups, only those receiving flaxseed lignan SDG showed a significant increase in total polyphenol levels in both healthy and MS rats when compared with the control and MS groups. As mentioned earlier, SDG is a lignan found in large amounts in flaxseed. Knowing that lignans are an important group of polyphenols, we can say that the increase in polyphenol levels is due to the presence of this compound in the bodies of animals that received SDG (Gharras 2009). However, although flaxseed is rich in polyphenols, it is known that they are mainly found in the seed coat and not in the embryo, from which the oil is extracted (Wiesenborn and others 2003). Thus, FO exhibits minimal polyphenols and thus its content was not changed in the FO-supplemented groups.

In addition to diet-derived antioxidants, the body is capable of producing endogenous antioxidants to minimize the damaging action of ROS. Endogenous antioxidants include GSH and some enzymes, such as SOD, CAT, and GPx (Halliwell and Gutteridge, 2015). GSH is the major nonprotein thiol in many living cells and is found in high concentrations in the body. The drastic reduction in GSH levels observed in the MS group compared with the control group was due to the conversion of GSH into GSSG, which occurs to protect cells from oxidative damage (Guo and others 2012). MS+FO and MS+SDG groups had significantly higher GSH levels than the MS group, demonstrating the protective role of both against GSH oxidation. This result also suggests the involvement of oxidative stress in the pathophysiology of MS induced by fructose-rich diets, since in healthy rats only the SDG-treated group showed an increase in GSH levels when compared with the control group. Furthermore, groups that received SDG demonstrated grater increases in GSH that those receiving FO, presenting even higher levels than the controls. This result demonstrates that SDG can increase GSH above normal levels, even in cases of high oxidative damage. However, the protective mechanism of FO and SDG against GSH depletion should be studied further.

Regarding antioxidant enzymes, the reduction in SOD and GPx activities in the MS group indicate that the increased oxidative damage observed in this group is due, at least in part, to a reduction in antioxidant status, although there is no consensus as to the exact mechanism by which MS affects antioxidant enzyme activities (Poudyal and others 2011). However, no significant differences were observed in CAT activity between the control and MS groups, which is in line with the findings of Bagul and others (2012), who also found no differences in this enzyme activity in animals fed a high-fructose diet compared with those fed a standard diet. Furthermore, the increase in activities of these 3 enzymes in groups that received FO and SDG shows that both substances can positively affect enzymatic antioxidant defenses both in healthy and MS groups, which is consistent with findings from previous studies (Tayyebi-Khosroshahi and others 2010; Pilar and others 2014; Zuravski and others 2015; Sawant and Bodhankar 2016). However, we observed a greater increase in groups treated with SDG, demonstrating the greater antioxidant power of this component.

Conclusion

This study was the only one to perform a comparison of the abilities of 2 components of flaxseed to protect against oxidative stress in a MS model. The findings of this study indicated an involvement of oxidative stress in the pathophysiology of MS induced by fructose-rich diets. Furthermore, we demonstrated that the antioxidant effects attributed to flaxseed are mainly due to its high lignan content, especially that of SDG, suggesting that this compound can be used in isolation to prevent oxidative stress associated with MS. Because FO has also shown protective effects against oxidative stress, we suggest that flaxseed can be more effective if ingested in its intact form, containing all the compounds. However, a study with all 3 feeding material (FO, SDG lignan and flaxseed) should be performed in the future.

Author Contributions

All authors participated in the design, interpretation of the studies and analysis of the data and review of the manuscript; B. Pilar, A. Güllich, P. Oliveira, D. Ströher and J. Piccoli conducted the experiments, and B. Pilar and V. Manfredini wrote the manuscript.

Conflict of Interest

The authors report no conflicts of interest.

References

Aebi H. 1984. Catalase in vitro. Methods Enzymol 105:121–6.

Akerboom TP, Sies H. 1981. Assay of glutathione, glutathione disulfide, and glutathione mixed disulfides in biological samples. Methods Enzymol 77:373–82.

Andersen CJ, Fernandez ML. 2013. Dietary strategies to reduce metabolic syndrome. Rev Endocr Metab Disord 14:241–54.

Armutcu F, Ataymen M, Atmaca H, Gurel A. 2008. Oxidative stress markers, C-reactive protein and heat shock protein 70 levels in subjects with metabolic syndrome. Clin Chem Lab Med 46:785–90.

Badawy EA, Rasheed WI, Elias TR, Hussein J, Harvi M, Morsy S, El-Latif Mahmoud YA. 2015. Flaxseed oil reduces oxidative stress and enhances brain monoamines release in streptozotocin-induced diabetic rats. Hum Exp Toxicol 34:1133–8.

Bagul PK, Middela H, Matapally S, Padiya R, Bastia T, Madhusudana K, Reddy BR, Chakravarty S, Banerjee SK. 2012. Attenuation of insulin resistance, metabolic syndrome and hepatic oxidative stress by resveratrol in fructose-fed rats. Pharmacol Res 66:260–8.

Barrera G. 2012. Oxidative stress and lipid peroxidation products in cancer progression and therapy. ISRN Oncol 2012:1–21.

Elnakish MT, Hassanain HH, Janssen PM, Angelos MG, Khan M. 2013. Emerging role of oxidative stress in metabolic syndrome and cardiovascular diseases: important role of Rac/NADPH oxidase. J Pathol 231:290–300.

Gharras HE. 2009. Polyphenols: food sources, properties and applications—a review. Intl J Food Sci Tech 44:2512–8.

Guermouche B, Soulimane-Mokhtari NA, Bouanane S, Merzouk H, Merzouk S, Narce M. 2014. Effect of dietary polyunsaturated fatty acids on oxidant/antioxidant status in macrosomic offspring of diabetic rats. Biomed Res Intl 2014:1–9.

Guimaraes PS, Oliveira MF, Braga JF, Nadu AP, Schreihofer A, Santos RAS, Campagnole-Santos MJ. 2014. Increasing angiotensin-(1–7) levels in the brain attenuates metabolic syndrome–related risks in fructose-fed rats. Hypertension 63:1078–85.

Guo Y, Wang H, Sun Y, Qu B. 2012. A disulfide bound-molecular beacon as a fluorescent probe for the detection of reduced glutathione and its application in cells. Chem Commun 48:3221–3.

Halliwell B, Gutteridge JMC. 2015. Free radicals in biology and medicine. 5th ed. Oxford: Oxford University Press.

Hu C, Yuan YV, Kitts DD. 2007. Antioxidant activities of the flaxseed lignan secoisolariciresinol diglucoside, its aglycone secoisolariciresinol and the mammalian lignans enterodiol and enterolactone in vitro. Food Chem Toxicol 45:2219–27.

Hutcheson R, Rocic P. 2012. The metabolic syndrome, oxidative stress, environment, and cardiovascular disease: the great exploration. Exp Diabetes Res 2012:1–13.

Kaithwas G, Majumdar DK. 2012. In vitro antioxidant andin vivo antidiabetic, antihyperlipidemic activity of linseed oil against streptozotocin-induced toxicity in albino rats. Eur J Lipid Sci Tech 114:12–45.

Kajla P, Sharma A, Sood DR. 2015. Flaxseed – a potencial functional food source. J Food Sci Technol 52:1857–71.

Karaka S, Eraslan G. 2013. The effects of flaxseed oil on cadmium-induced oxidative stress in rats. Biol Trace Elem Res 155:423–30.

Kassi E, Pervanidou P, Kaltsas G, Chrousos G. 2011. Metabolic syndrome: definitions and controversies. BMC Med 9:48.

Khalesi S, Jamaluddin R, Ismail A. 2011. Effect of raw and heated flaxseed (*Linum usitatissimum* L.) on blood lipid profiles in rats. Intl J Appl Sci Technol 1:84–9.

Leibowitz A, Rehman A, Paradis P, Schiffrin EL. 2013. Role of T regulatory lymphocytes in the pathogenesis of high-fructose diet-induced metabolic syndrome. Hypertension 61: 1316–21.

Levine ER, Garland D, Oliver CN, Amici A, Climent I, Lenz AG, Ahn BW, Shaltiel S, Stadtman ER. 1990. Determination of carbonyl content in oxidatively modified proteins. Methods Enzymol 186:464–78.

Newaury AA, Abdou HM. 2009. Protective role of flax lignans against lead acetate induced oxidative damage and hyperlipidemia in rats. Food Chem Toxicol 47:813–8.

Ogawa A, Suzuki Y, Aoyama T, Takeuchi H. 2009. Dietary alpha-linolenic acid inhibits angiotensin-converting enzyme activity and mRNA expression levels in the aorta of spontaneously hypertensive rats. J Oleo Sci 58:355–60.

Ohkawa H, Ohishi N, Yagi K. 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 95:351–8.

Pandey KB, Mishra N, Rizvi SI. 2010. Protein oxidation biomarkers in plasma of type 2 diabetic patients. Clin Biochem 43:508–11.

Park DY, Ahn YT, Huh CS, McGregor RA, Choi M. 2013. Dual probiotic strains suppress high fructose-induced metabolic syndrome. World J Gastroenterol 19:274–83.

Park JB, Velasquez MT. 2012. Potential effects of lignan-enriched flaxseed powder on bodyweight, visceral fat, lipid profile, and blood pressure in rats. Fitoterapia 83:941–6.

Pattyn N, Cornelissen VA, Eshghi SRT, Vanhees L. 2013. The effect of exercise on the cardiovascular risk factors constituting the metabolic syndrome. Sports Med 43:121–33.

Penumathsa SV, Koneru S, Zhan L, John S, Menon VP, Prasad K, Maulik N. 2008. Secoisolariciresinol diglucoside induces neovascularization-mediated cardioprotection against ischemia–reperfusion injury in hypercholesterolemic myocardium. J Mol Cell Cardiol 44: 170–9.

Pérez-Jiménez J, Díaz-Rubio ME, Saura-Calixto F. 2015. Contribution of macromolecular antioxidants to dietary antioxidant capacity: a study in the spanish mediterranean diet. Plant Foods Hum Nutr 70:365–70.

Pilar BC, Güllich AAC, Ströher DJ, Zuravski L, Mezzomo J, Coelho RP, Faoro D, Piccoli JCE, Manfredini V. 2014. 28-days dietary supplementation with golden flaxseed improves biochemical and oxidative parameters in patients with metabolic syndrome. J Funct Foods 10:232–42.

Poudyal H, Panchal SK, Diwan V, Brown L. 2011. Omega-3 fatty acids and metabolic syndrome: effects and emerging mechanisms of action. Prog Lipid Res 50:372–87.

Reuter S, Gupta SC, Chaturvedi MM, Aggarwal BB. 2010. Oxidative stress, inflammation, and cancer: how are they linked?. Free Radic Biol Med 49:1603–16.

Rodriguez-Leyva D, Bassett CM, McCullough R, Pierce GN. 2010. The cardiovascular effects of flaxseed and its omega-3 fatty acid, alpha-linolenic acid. Can J Cardiol 26: 489–96.
Roncal CA, Reungjui S, Sánchez-Lozada LG, Mu W, Sautin YY, Nakagawa T, Johnson RJ. 2009. Combination of captopril and allopurinol retards fructose-induced metabolic syndrome. Am J Nephrol 30:399–404.
Rubilar M, Gutiérrez C, Verdugo M, Shene C, Sineiro J. 2010. Flaxseed as a source of functional ingredientes. J Soil Sci Plant Nutr 10:373–7.
Sawant SH, Bodhankar SL. 2016. Flax lignan concentrate ameliorates Nω-nitro-L-arginine methyl ester (L-NAME)-induced hypertension: role of antioxidant, angiotensin-converting enzyme and nitric oxide. Pharmacologia 7:157–69.
Schmid W. 1975. The micronucleus test. Mutat Res 31:9–15.
Singh NP, McCoy MT, Tice RR, Schneider EL. 1988. A simple technique for quantitation of low levels of DNA damage in individual cells. Exp Cell Res 175:184–91.
Singleton VL, Orthofer R, Lamuela-Raventos RM. 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol 299:152–78.
Tamarit J, Hoogh A, Obis E, Alsina D, Cabiscol E, Ros J. 2012. Analysis of oxidative stress-induced protein carbonylation using fluorescent hydrazides. J Proteom 75:3778–88.
Taylor CG, Noto AD, Stringer DM, Froese S, Malcolmson L. 2010. Dietary milled flaxseed and flaxseed oil improve N-3 fatty acid status and do not affect glycemic control in individuals with well-controlled type 2 diabetes. J Am Coll Nutr 29:72–80.
Tayyebi-Khosroshahi H, Houshyar J, Tabrizi A, Vatankhah A, Zonouz NR, Dehghan-Hesari R. 2010. Effect of omega-3 fatty acid on oxidative stress in patients on hemodialysis. Iran J Kidney Dis 4:322–6.
Tzang B, Yang S, Fu S, Yang H, Sun H, Chen Y. 2009. Effects of dietary flaxseed oil on cholesterol metabolism of hamsters. Food Chem 114:1450–5.
Wiesenborn D, Tostenson K, Kangas N. 2003. Continuous abrasive method for mechanically fractionating flaxseed. J Am Oil Chem Soc 80:295–300.
Zhang W, Wang X, Liu Y, Tian H, Flickinger B, Empie MW, Sun SZ. 2008. Dietary flaxseed lignan extract lowers plasma cholesterol and glucose concentrations in hypercholesterolaemic subjects. Br J Nutr 99:1301–9.
Zuravski L, Coelho RP, Duarte JA, Rocha MA, Mezzomo J, Pilar BC, Galarça LL, Athayde ML, Boligon AA, Machado MM, Manfredini V. 2015. protective role of golden flaxseed (*Linum usitatissimum* L.) against oxidative damage in lipids and proteins of healthy volunteers. J Biosci Med 3:45–53.

5 MANUSCRITO

Effect of flaxseed oil and flaxseed lignan secoisolariciresinol diglucoside in preventing metabolic syndrome and its complications in rats

Submetido ao periódico "Nutrition & Metabolism"

CONFIRMAÇÃO DE SUBMISSÃO

De: Nutrition & Metabolism Editorial Office <em@editorialmanager.com>
Para: Bruna Pilar (brunapilar16@hotmail.com)
Assunto: Confirmation of your submission to Nutrition & Metabolism NUAM-D-17-00212

14-Set-2017

NUAM-D-17-00212
Effect of flaxseed oil and flaxseed lignan secoisolariciresinol diglucoside in preventing metabolic syndrome and its complications in rats
Bruna Pilar, M.D.; Angélica Aparecida da Costa Güllich, Master; Juliana Mezzomo, Master; Ritiele Pinto Coelho, Master; Jacqueline da Costa Escobar Piccoli, Doctor; Vanusa Manfredini
Nutrition & Metabolism

Dear Ms. Pilar,

Thank you for submitting your manuscript 'Effect of flaxseed oil and flaxseed lignan secoisolariciresinol diglucoside in preventing metabolic syndrome and its complications in rats' to Nutrition & Metabolism.

The submission id is: NUAM-D-17-00212
Please refer to this number in any future correspondence.

During the review process, you can keep track of the status of your manuscript by accessing the following website:

http://nuam.edmgr.com/

Your username is: brunapilar16@hotmail.com
If you forgot your password, you can click the 'Send Login Details' link on the EM Login page at http://nuam.edmgr.com/.

Best wishes,
Editorial Office
Nutrition & Metabolism
https://nutritionandmetabolism.biomedcentral.com/

Effect of flaxseed oil and flaxseed lignan secoisolariciresinol diglucoside in preventing metabolic syndrome and its complications in rats

Bruna Cocco Pilar[a]*, Angélica Aparecida da Costa Güllich[a], Juliana Mezzomo[a], Ritiele Pinto Coelho[a], Jacqueline da Costa Escobar Piccoli[b], Vanusa Manfredini[a]

[a]Postgraduate Program in Biochemistry, Federal University of Pampa, Uruguaiana, Rio Grande do Sul, Brazil.

[b]Postgraduate Program in Pharmaceutical Sciences, Federal University of Pampa, Uruguaiana, Rio Grande do Sul, Brazil.

Authors' e-mail adress:

Bruna Cocco Pilar - brunapilar16@hotmail.com; Angélica Aparecida da Costa Güllich - angelicagullich@hotmail.com; Juliana Mezzomo - mezzomoj@yahoo.com.br; Ritiele Pinto Coelho - rity_coelho@hotmail.com; Jacqueline da Costa Escobar Piccoli - jcepiccoli@yahoo.com.br; Vanusa Manfredini - vanusa_manfredini@yahoo.com.br.

***Corresponding author:**

Universidade Federal do Pampa (UNIPAMPA) - Campus Uruguaiana

BR 472, Km 585 - Laboratório de Hematologia e Citologia Clínica

Uruguaiana, RS, Brazil, 97500-970

Telephone: +55 55 3911 0200

E-mail: brunapilar16@hotmail.com

Abstract

Background: Evidences has shown a positive role for flaxseed in the treatment and prevention of metabolic syndrome (MS), but there is no consensus on the component responsible for such effect. To clarify this, the present study evaluated the effects of FO and flaxseed lignan SDG in preventing metabolic syndrome and its complications in rats. **Methods:** A total of 48 Wistar rats were allocated into the following six groups. Groups I (control), V (FO) and VI (SDG) received water *ad libitum*, and groups II (MS), III (MS+FO) and IV (MS+SDG) received 30% fructose in drinking water for MS induction. Concomitantly, the animals received saline (groups I and II), FO (groups III and V), and SDG (groups IV and VI) by oral gavage. After 30 days, the animals were sacrificed, and blood, liver and kidneys were collected for biochemical, inflammatory and histopathological analyses. Body weight was recorded weekly. Systolic blood pressure was measured before and after treatment. **Results:** Fructose induced MS, besides promoting renal and inflammatory changes. FO and SDG prevented MS development as well as renal and inflammatory changes. **Conclusions:** These results demonstrated that treatment with 30% fructose solution for 30 days is an effective model for MS induction in rats. Furthermore, FO and SDG supplementation could prevent similarly blood pressure, biochemical and inflammatory changes observed in this disorder, which demonstrated that both could prevent the MS development in rats.

Keywords: flaxseed oil; secoisolariciresinol diglucoside; metabolic syndrome; rats; biochemistry; inflammation.

1 Background

Food choices and eating behavior are increasingly discussed in the health area. This concern has promoted an increase in the search for foods by consumers that besides their basic properties possess health benefits, the so-called functional foods. This new conduct has brought potential benefits for consumers' diet and new business opportunities for producers [1, 2].

Flaxseed (*Linum usitatissimum* L.) is a food that is establishing importance in the world's food chain as a functional food. Its excellent nutritional profile is due to its composition rich in alpha-linolenic acid (ALA), lignans, high quality proteins and soluble fibers [3]. In oil form, flaxseed contains approximately 57% ALA, being the richest source of ALA. It has been shown in previous studies that eicosapentaenoic acid and docosahexaenoic acid, which are formed from ALA metabolism, could reduce the risk of chronic diseases, such as atherosclerosis, cardiovascular disease, cancer and hyperlipidemia [4]. Furthermore, flaxseed contains up to 800 times more lignans than those in other plant foods, and its lignan content primarily consists of secoisolariciresinol diglucoside (SDG) (294–700 mg/100g) [5]. After its metabolism in the intestine and colon, SDG is broken down into secoisolariciresinol, enterodiol and enterolactone molecules, which have already shown health beneficial effects in the prevention and as an adjunct in the treatment of chronic diseases such as hypercholesterolemia and diabetes mellitus [6, 7, 8].

The term metabolic syndrome (MS) has been used by researchers to define a set of risk factors of metabolic origin, which is considered as a major and escalating public health and clinical challenge worldwide due to surplus energy intake and sedentary lifestyle habits [9]. According to a later definition proposed by the International Diabetes Federation (IDF), MS corresponds to a combination of at least three risk factors, including visceral obesity,

hypertension, increased triglyceride and glucose levels, and decreased high-density lipoprotein (HDL) cholesterol levels [10]. Furthermore, studies have associated MS with a pro-inflammatory state and hepatic and renal changes [11, 12, 13]. The treatment of MS is complex and includes changes in lifestyle and drug treatment of risk factors [14]. Furthermore, studies have indicated that an increased intake of flaxseed or its isolated components is inversely related to the presence of MS characteristics [8, 15-17].

Although evidence shows a positive role for flaxseed in the treatment and prevention of MS, there is no consensus on the component responsible for such effect. Some studies attribute these effects to flaxseed oil (FO) and its high ALA concentration [18, 19], whereas other studies indicate that the health effects of flaxseed in MS are due to the presence of its lignans, especially SDG [8, 20]. Therefore, the present study evaluated the effects of FO and flaxseed lignan SDG in preventing metabolic syndrome and its complications in rats.

2 Material and Methods

2.1 Chemicals

All the chemicals used were of analytical grade. All reagents and lignan SDG were obtained from Sigma Chemical Co. (St. Louis, MO, USA). FO was acquired from Mundo dos Óleos Company (Brasilia, DF, Brazil). According to the manufacturers' information, FO was obtained by cold pressing and filtration from seeds and SDG presented a purity $\geq 97\%$ (high-performance liquid chromatography).

2.2 Animals and diets

Male Wistar rats (30 days old) were purchased from the Central Animal Laboratory of University Federal of Santa Maria (Santa Maria, RS, Brazil). The animals were housed in cages under controlled conditions with 12 h light and 12 h dark cycle (temperature, 22 ± 1 °C; humidity, 55 ± 5%) and fed with standard laboratory chow and water *ad libitum*.

Rats were randomly allocated into six groups (eight rats per group). Groups II, III and IV received 30% fructose solution in drinking water during the experimental period (30 days) for MS induction, while groups I, V and VI received water *ad libitum*. Simultaneously, the animals received daily treatment by oral gavage as follows: Groups I and II (control and MS groups, respectively): 1 mL/kg body weight saline; Groups III and V (MS + FO and FO groups): 1 mL/kg body weight FO [19]; and Groups IV and VI (MS + SDG and SDG groups): 20 mg/kg body weight SDG [20];

At the end of the experiment, the rats were anesthetized and euthanized by decapitation, and whole blood was collected into a clean tube for serum separation. In addition, the liver and kidneys were collected.

2.3 Body weight and systolic blood pressure

Body weight was recorded weekly. Systolic blood pressure (SBP) was measured by non-invasive tail-cuff plethysmography (AD Instruments Pty Ltd, Bella Vista, NSW, Australia) before and after treatment in conscious rats. Before measurement, the rats were kept at 30°C for 10 min to make the pulsations of the tail artery detectable. To establish the SBP value, 10 measurements were taken, and the mean was obtained. To guarantee the reliability of the measurements, we established a training period of 1 week before the actual trial time, and during this period, the rats were accustomed to the procedure. Moreover, to

minimize stress-induced variations in blood pressure, all measurements were taken by the same person in the same quiet environment.

2.4 Biochemical and inflammatory analyses

Lipid profile was analyzed by determination of total cholesterol, HDL cholesterol, and triglyceride levels. Furthermore, the levels of glucose, creatinine, and blood urea nitrogen (BUN), and the activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured using the A25 Biosystems automatic analyzer (Barcelona, Spain) for *in vitro* diagnostics. Homocysteine levels were measured by high-performance liquid chromatography coupled to mass spectrometry (LC-MS/MS), according to Nelson et al. [21].

White blood cell (WBC) count was performed using the automated hematology analyzer Sysmex KX-21N (Kobe, Japan). Adiponectin concentrations were measured using a commercially available competitive enzyme immunoassay (ELISA) kit (Abnova Corporation, Walnut, CA, USA). Interleukin-6 (IL-6) levels were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Enzo Life Sciences Inc., Farmingdale, NY, USA). All assays were carried out in triplicate.

2.5 Histopathological analysis

Liver and kidneys were fixed by immersion at room temperature in 10% (v/v) formalin solution. For histopathological examinations, paraffin-embedded tissue sections (4mm) were stained with hematoxylin–eosin (H&E) and examined for observation of structural abnormalities. Images were made from photographs taken with a 10-MP camera attached to a Leica DM500 microscope (installed with the LAS EZ-V 3.4.0 software).

2.6 Statistical analysis

Data were expressed as mean ± standard deviation (SD). Data were analyzed by one-way analysis of variance (ANOVA) followed by Bonferroni test for multiple comparisons. P values < 0.05 were considered as statistically significant. Statistical analyses were performed using GraphPad Prism software (version 5.0, GraphPad Software, Inc., La Jolla, CA).

3 Results

3.1 Body weight and SBP

The group that received fructose solution (MS) had a percentage increase in SBP significantly higher ($p < 0.05$) than in the control group, and the treatment with FO and SDG prevented this alteration. In healthy rats, FO and SDG did not cause significant changes in SBP increase when compared to the control group (Figure 1). There was no significant difference in body weight gain between the groups during the treatment period (data not shown).

3.2 Biochemical and inflammatory analyses

Table 1 shows the results for lipid profile, fasting glucose, and renal, hepatic, and inflammatory markers of different groups. Treatment with 30% fructose solution for 30 days significantly increased ($p < 0.05$) the levels of glucose, triglycerides, total cholesterol, creatinine, BUN, homocysteine, and IL-6, and AST activity and WBC count. Furthermore,

this treatment resulted in a statistically significant reduction ($p < 0.05$) in the levels of HDL cholesterol and adiponectin compared to those in the control group.

Treatment with FO and SDG significantly prevented ($p < 0.05$) the increase in the levels of triglycerides, total cholesterol, creatinine, BUN, homocysteine, and IL-6 and AST activity and WBC count. In addition, these compounds significantly prevented ($p < 0.05$) the reduction in the levels of HDL cholesterol and adiponectin observed in the MS group. In glucose levels only SDG prevented the increase caused by the fructose solution. Regarding triglycerides and HDL cholesterol levels and AST activity, FO had a better protective effect than that with SDG. However, SDG demonstrated more pronounced protective effects in the levels of BUN, homocysteine, IL-6, and adiponectin and WBC count. For the other parameters, the prevention was similar between the groups treated with FO and SDG. Regarding ALT activity, there were no significant differences among the groups.

In healthy rats, FO group presented significantly lower values ($p < 0.05$) only in BUN levels and WBC count when compared to the control group. SDG group presented statistically lower values only in WBC count. In other parameters, no significant differences were observed between the FO and SDG treated groups and the control group.

Table 1: Biochemical and inflammatory parameters in different study groups.

	Control	**MS**	**MS + FO**	**MS + SDG**	**FO**	**SDG**
Glucose (mg/dL)	109.7 ± 20.7	151.6 ± 14.8[a]	144.0 ± 10.8[a]	107.8 ± 10.6[b]	115.6 ± 10.6[b]	104.6 ± 17.9[b]
Triglycerides (mg/dL)	107.7 ± 10.3	312.8 ± 2.5[a]	172.8 ± 30.3[a,b]	196.0 ± 65.7[a,b]	110.8 ± 14.5[b]	108.2 ± 10.9[b]
Total Cholesterol (mg/dL)	79.3 ± 4.1	208.3 ± 12.9[a]	71.6 ± 4.4[b]	79.4 ± 20.7[b]	89.9 ± 11.9[b]	90.8 ± 12.4[b]
HDL Cholesterol (mg/dL)	48.3 ± 0.4	24.4 ± 1.9[a]	41.5 ± 5.2[a,b]	31.3 ± 1.6[a,b]	49.3 ± 5.5[b]	47.1 ± 1.8[b]
Creatinine (mg/dL)	0.33 ± 0.02	0.66 ± 0.03[a]	0.57 ± 0.06[a,b]	0.55 ± 0.04[a,b]	0.30 ± 0.03[b]	0.31 ± 0.05[b]
BUN (mg/dL)	38.5 ± 0.5	53.4 ± 2.0[a]	32.4 ± 5.3[a,b]	23.0 ± 5.5[a,b]	28.7 ± 4.3[a,b]	34.5 ± 2.7[b]
Homocystheine (µmol/L)	5.9 ± 0.2	23.4 ± 0.5[a]	15.1 ± 0.2[a,b]	13.1 ± 0.2[a,b]	5.7 ± 0.1[b]	5.7 ± 0.3[b]
ALT (U/L)	66.0 ± 6.4	68.0 ± 9.1	63.3 ± 7.7	70.5 ± 0.5	58.1 ± 8.6[b]	65.0 ± 9.6
AST (U/L)	132.9 ± 33.0	282.4 ± 63,5[a]	179.7 ± 16.1[a,b]	235.0 ± 25.5[a,b]	117.5 ± 25.4[b]	123.6 ± 43.1[b]
WBC (10^3/µL)	15.3 ± 3.3	18.5 ± 1.8[a]	10.9 ± 2.7[a,b]	10.4 ± 2.0[a,b]	12.3 ± 1.3[a,b]	12.7 ± 2.7[a,b]
IL-6 (pg/mL)	11.3 ± 1.1	32.5 ± 2.8[a]	25.7 ± 1.9[a,b]	17.4 ± 1.1[a,b]	12.6 ± 2.9[b]	11.7 ± 2.9[b]
Adiponectin (µg/mL)	12.4 ± 0.6	8.2 ± 0.4[a]	9.8 ± 0.3[a,b]	11.6 ± 0.7[a,b]	11.1 ± 0.5[b]	12.2 ± 0.3[b]

Values expressed as mean ± SD. a: $p < 0.05$ compared with control group. b: $p < 0.05$ compared with MS group.

3.3 Histopathological analysis

Histopathological analysis of the livers and kidneys revealed no structural differences between the groups (Figures 2 and 3, respectively).

4 Discussion

Diets with high amounts of fructose (10-60%) have already been standardized by several researchers for the induction of MS [22, 23]. In our study, we induced MS by treatment with 30% fructose solution in drinking water for 30 days. This solution promoted an increase in SBP and glucose and triglyceride levels and reduced HDL cholesterol levels, demonstrating that this is an effective model for MS induction in Wistar rats.

The protective effects of FO and SDG on the increase in SBP observed in this study are in agreement with other studies [24-26]. The study of Ogawa et al. [24] demonstrated a lower activity and reduced expression of mRNA of angiotensin converting enzyme (ACE) in the ALA-supplemented group, suggesting that SBP reduction mechanism in groups supplemented with FO involves the reduction of ACE levels in the aorta. In addition, the study by Prasad [25] demonstrated that SDG reduced the angiotensin I-induced rise in the arterial pressures, and hence, SDG could act as a potent ACE inhibitor.

Regarding lipids levels, the results of this study demonstrated that FO and SDG supplementation prevented the increase in the levels of triglycerides and total cholesterol, and the reduction in HDL cholesterol levels caused by fructose. In addition, SDG prevented the increase in glucose levels observed in MS. These findings confirm the results of previous studies that have shown that FO and flaxseed lignans could prevent the increase in blood

glucose and blood lipid levels in hypercholesterolemia and diabetes [19, 27, 28, 29, 30]. These studies suggest that the reduction in glucose levels by SDG is due to its ability to enhance the transport of blood glucose to peripheral tissues, reducing its levels in plasma[(30)]. With respect to lipid levels, studies indicate that ALA can activate genes involved in fatty acid metabolism, reducing its synthesis [29], and SDG can reduce HMG-CoA activity, reducing cholesterol synthesis, besides decreasing the lipolysis rate [30]. These results demonstrated that although FO had better results in improving HDL levels and SDG was better at preventing increased glucose levels, both could contribute to inhibition of the development of MS induced by a fructose-rich diet.

Studies have shown that MS is associated with an increased risk of kidney damage [12]. In this study, we observed an increase in the levels of classic markers of kidney function, BUN, and creatinine after fructose supplementation, indicating that MS factors are indeed associated with renal dysfunction. In addition, FO and SDG supplementation could prevent these changes, which is in line with previous studies [31, 32]. However, Gluba et al. [12] showed that these lesions are related to individual risk factors such as hypertension and abnormal levels of glucose, triglycerides, and HDL cholesterol and not with MS as a whole, indicating that the positive effects of FO and SDG on renal markers are related to the improvement in these individual parameters and not with MS as a whole. In addition, hyperhomocysteinemia is a common finding in patients with kidney disease, indicating that the significant increase in homocysteine levels observed in the MS group compared to that in the control group is also related to the association of MS with renal damage [33]. An already significant reduction in the levels of this marker in the FO- and SDG-supplemented groups confirms their role in preventing such damage. However, despite the changes observed in these markers, the histopathological analysis revealed no apparent tissue changes, probably due to the short treatment period.

Several authors have also indicated an association between high-fructose diets and non-alcoholic fatty liver disease (NAFLD) due to increased visceral adiposity caused by fructose [13]. In this study, we observed a significant increase in AST activity in the MS group, although no significant differences were found in ALT activity and liver histopathology. Knowing that changes in AST levels are less common in these disorders compared to changes in ALT levels and liver histopathology, we can say that, although the protocol used in this study induced MS, it could not promote hepatic changes related to NAFLD [34]. In addition, although FO and SDG partially prevented the increase in AST activity caused by fructose solution, no significant differences in ALT activity and liver structure were observed in comparison with the control and MS groups, indicating that these compounds can not cause liver changes. However, other studies using longer treatment periods are necessary to confirm this statement.

Furthermore, it is well known that individuals with MS often have a pro-inflammatory state with increased levels of interleukins, WBC count, coagulation factors, and C-reactive protein, as well as decreased levels of adiponectin [11]. In this study, the inflammatory state was evaluated through the WBC count and levels of IL-6, an inflammatory cytokine involved in several biological processes, and adiponectin, an adipokine secreted by adipose tissue with anti-inflammatory and insulin-sensitizing activities [35, 36]. The increase in WBC count and IL-6 levels associated with the reduction in adiponectin levels observed in the MS group indicates the presence of an inflammatory process associated with this disorder. In addition, FO and SDG reduced WBC count and IL-6 levels and increased adiponectin levels, indicating that both FO and SDG could prevent MS-associated inflammation, with SDG showing better effects. Dallmeier et al. [37] suggested that the relationship between MS and inflammatory markers is largely explained by the individual components of MS, indicating that although no significant differences were observed in the body weight of the rats in this study, alteration of

these inflammatory markers may be related to deregulation of serum lipid and glucose levels and blood pressure. In addition, it is suggested that the improvement in these parameters in the groups treated with FO and SDG would also explain the reduction in inflammation in these groups, especially in the SDG group.

Since in healthy rats no significant differences were observed in most parameters between the FO- and SDG-supplemented groups and the control group, we can suggest that the beneficial effect of these compounds is related with the presence of MS.

5 Conclusions

The results of this study demonstrated that treatment with 30% fructose solution for 30 days is an effective model for MS induction in Wistar rats. Furthermore, FO and SDG supplementation prevented blood pressure and biochemical and inflammatory changes observed in this disorder, which demonstrated that both could prevent the MS development in rats.

List of abbreviations

ACE, angiotensin converting enzyme; ALA, alpha-linolenic acid; ALT, alanine aminotransferase; ANOVA, one-way analysis of variance; AST, aspartate aminotransferase; BUN, blood urea nitrogen; FO, flaxseed oil; IDF, international diabetes federation; IL-6, Interleukin-6; HDL, high density lipoprotein; H&E, hematoxylin–eosin; MS, metabolic syndrome; NAFLD, non-alcoholic fatty liver disease; SBP, systolic blood pressure; SD, standard deviation; SDG, secoisolariciresinol diglucoside; WBC, white blood cell.

Declarations

Ethics approval and consent to participate

The experiments were ethically approved by the Ethics Committee on Animal Use of the Federal University of Pampa (Uruguaiana, RS, Brazil) (Protocol 024/2014).

Consent for publication

Not applicable

Availability of data and material

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Authors' contributions

All authors participated in the design, interpretation of the studies and analysis of the data and review of the manuscript; BCP, AACG, PMO, DS and JP conducted the experiments, and BP and VM wrote the manuscript.

Acknowledgements

Not applicable

REFERENCES

[1] WHO. Global status report on noncommunicable diseases. 1st ed. Switzerland: WHO Press; 2011.

[2] Annunziata A, Vecchio R. Consumer perception of functional foods: A conjoint analysis with probiotics. Food Qual Prefer 2013;28:348-55

[3] Kajla P, Sharma A, Sood DR. Flaxseed – a potencial functional food source. J Food Sci Technol 2015;52:1857-71.

[4] Rodriguez-Leyva D, Bassett CM, McCullough R, Pierce GN. The cardiovascular effects of flaxseed and its omega-3 fatty acid, alpha-linolenic acid. Can J Cardiol 2010;26:489-496.

[5] Goyal A, Sharma V, Upadhyay N, Gill S, Sihag M. Flax and flaxseed oil: an ancient medicine & modern functional food. J Food Sci Technol 2014;51:1633-53.

[6] Hu C, Yuan YV, Kitts DD. Antioxidant activities of the flaxseed lignan secoisolariciresinol diglucoside, its aglycone secoisolariciresinol and the mammalian lignans enterodiol and enterolactone in vitro. Food Chem Toxicol 2007;45:2219–27.

[7] Pan A, Sun J, Chen Y, Ye1 X, Li H, Yu Z, et al. Effects of a flaxseed-derived lignan supplement in type 2 diabetic patients: a randomized, double-blind, cross-over trial. PLoS One 2007;2:e1148.

[8] Zhang W, Wang X, Liu Y, Tian H, Flickinger B, Empie MW, et al. Dietary flaxseed lignan extract lowers plasma cholesterol and glucose concentrations in hypercholesterolaemic subjects. Br J Nutr 2008;99:1301-09.

[9] Kaur J. A comprehensive review on metabolic syndrome. Cardiol Res Pract 2014;1-21.

[10] Kassi E, Pervanidou P, Kaltsas G, Chrousos, G. Metabolic syndrome: definitions and controversies. BMC Med 2011;9:48.

[11] Kraja AT, Chasman TI, North KE, Reiner AP, Yanek LR, Kilpeläinen TO, et al. Pleiotropic genes for metabolic syndrome and inflammation. Mol Genet Metab 2014;112:317-38.

[12] Gluba A, Mikhailidis DP, Lip GYH, Hannam S, Rysz J, Banach M. Metabolic syndrome and renal disease. Int J Cardiol 2012;164:141-50.

[13] Vos MB, Lavine JE. Dietary fructose in nonalcoholic fatty liver disease, Hepatology 2013;57:2525-30.

[14] López-Jaramillo P, Sánchez RA, Diaz M, Cobos L, Bryce A, Parra-Carrillo JZ, et al. Consenso latino-americano de hipertensão em pacientes com diabetes tipo 2 e síndrome metabólica. Arq Bras Endocrinol Metab 2014;58:205-25.

[15] Couto AN, Wichmann FMA. Efeitos da farinha de linhaça no perfil lipídico e antropométrico. Braz J Food Nutr 2011;22:601-8.

[16] Pilar BC, Güllich AAC, Ströher DJ, Zuravski L, Mezzomo J, Coelho RP, et al. 28-days dietary supplementation with golden flaxseed improves biochemical and oxidative parameters in patients with metabolic syndrome. J Funct Foods 2014;10:232-42.

[17] Rhee Y, Brunt A. Flaxseed supplementation improved insulin resistance in obese glucose intolerant people: a randomized crossover design. Nutr J 2011;10: 44-50.

[18] Amir K, Watanabe M, Yokotsuka M, Kobayashi Y. Changes in serum fatty acids and effects of metabolic syndrome induced by intermittent flaxseed-oil supplementation. Ningen Dock, 2012;26:927-34.

[19] Kaithwas G, Majumdar DK. In vitro antioxidant and in vivo antidiabetic, antihyperlipidemic activity of linseed oil against streptozotocin-induced toxicity in albino rats. Eur J Lipid Sci Tech 2012;114:1237-45.

[20] Penumathsa SV, Koneru S, Zhan L, John S, Menon VP, Prasad K, et al. Secoisolariciresinol diglucoside induces neovascularization-mediated cardioprotection against ischemia–reperfusion injury in hypercholesterolemic myocardium. J Mol Cell Cardiol 2008;44:170-9.

[21] Nelson BC, Pfeiffer CM, Sniegoski LT, Satterfield MB. Development and evaluation of an isotope dilution LC/MS method for the determination of total homocysteine in human plasma. Anal Chem 2003;75:775-84.

[22] Leibowitz A, Rehman A, Paradis P, Schiffrin, EL, et al. Role of T regulatory lymphocytes in the pathogenesis of high-fructose diet - induced metabolic syndrome. Hypertension 2013;61:1316-21.

[23] Guimaraes PS, Oliveira MF, Braga JF, Nadu AP, Schreihofer A, Santos RAS, et al. Increasing angiotensin-(1–7) levels in the brain attenuates metabolic syndrome–related risks in fructose-fed rats. Hypertension 2014;63:1078-85.

[24] Ogawa A, Suzuki Y, Aoyama T, Takeuchi H. Dietary alpha-linolenic acid inhibits angiotensin-converting enzyme activity and mRNA expression levels in the aorta of spontaneously hypertensive rats. J Oleo Sci 2009;58:355-60.

[25] Prasad K. Secoisolariciresinol diglucoside (SDG) isolated from flaxseed, an alternative to ACE inhibitors in the treatment of hypertension. Int J Angiol 2013;22:235-8.

[26] Park JB, Velasquez MT. Potential effects of lignan-enriched flaxseed powder on body weight, visceral fat, lipid profile, and blood pressure in rats. Fitoterapia 2012;83:941-6.

[27] Fukumitsu S, Aida K, Shimizu H, Toyoda K. Flaxseed lignan lowers blood cholesterol and decreases liver disease risk factors in moderately hypercholesterolemic men. Nutr Res 2010;30:441-6.

[28] Akpolat M, Kanter M, Topcu-Tarladacalisir Y, Aydogdu N. Protective effect of flaxseed oil on renal injury in hyperlipidaemic rats: the effect of flaxseed oil on hyperlipidaemia. Phytother Res 2011;25:796-802.

[29] Devarshi P, Jangale NM, Ghule AE, Bodhankar SL, Harsulkar AM. Beneficial effects of flaxseed oil and fish oil diet are through modulation of different hepatic genes involved in lipid metabolismo in streptozotocin–nicotinamide induced diabetic rats. Genes Nutr 2013;8:329-42.

[30] Moree SS, Kavishankar GB, Rajesha J. Antidiabetic effect of secoisolariciresinol diglucoside in streptozotocin-induced diabetic rats. Phytomedicine 2013;20:237-45.

[31] Naqshbandi A, Rizwan S, Khan F. Dietary supplementation of flaxseed oil ameliorates the effect of cisplatin on rat kidney. J Funct Foods 2013;5:316-26.

[32] Sherif IO. Secoisolariciresinol diglucoside in high-fat diet and streptozotocin-induced diabetic nephropathy in rats: a possible renoprotective effect. J Physiol Biochem 2014;70:961-9.

[33] Shankar A, Wang JJ, Chua B, Rochtchina E, Flood V, Mitchell P. Positive association between plasma homocysteine level and chronic kidney disease. Kidney Blood Press Research 2008;31:55-62.

[34] Kotronen A, Yki-Järvinen H. Fatty liver: a novel component of the metabolic syndrome. Arterioscler Thromb Vasc Biol 2008;28:27-38.

[35] Rossi JF, Lu ZY, Jourdan M, Klein B. Interleukin-6 as a Therapeutic Target. Clin Cancer Res 2015;21:OF1-10.

[36] Hirose H, Yamamoto Y, Seino-Yoshihara Y, Kawabe H, Saito I. Serum high-molecular-weight adiponectin as a marker for the evaluation and care of subjects with metabolic syndrome and related disorders. J Atheroscler Thromb 2010;17:1201-11.

[37] Dallmeier D, Larson MG, Vasan RS, Keaney Jr JF, Fontes JD, Meigs JB, et al. Metabolic syndrome and inflammatory biomarkers: a community-based cross-sectional study at the Framingham Heart Study. Diabetol Metab Syndr 2012;4:28.

Figure legends

Fig. 1 Percent increase in systolic blood pressure during the experimental period. Data were analyzed by one-way analysis of variance (ANOVA) followed by Bonferroni test for multiple comparisons and expressed as mean ± SD. a: $p < 0.05$ compared to control group. b: $p < 0.05$ compared to MS group.

Fig. 2 Histopathological sections of livers. A: control group. B: MS group. C: MS + FO group. D: MS + SDG group. E: FO group. F: SDG group (H&E; 40X).

Fig. 3 Histopathological sections of kidneys. A: control group. B: MS group. C: MS + FO group. D: MS + SDG group. E: FO group. F: SDG group (H&E; 40X).

Figure 1

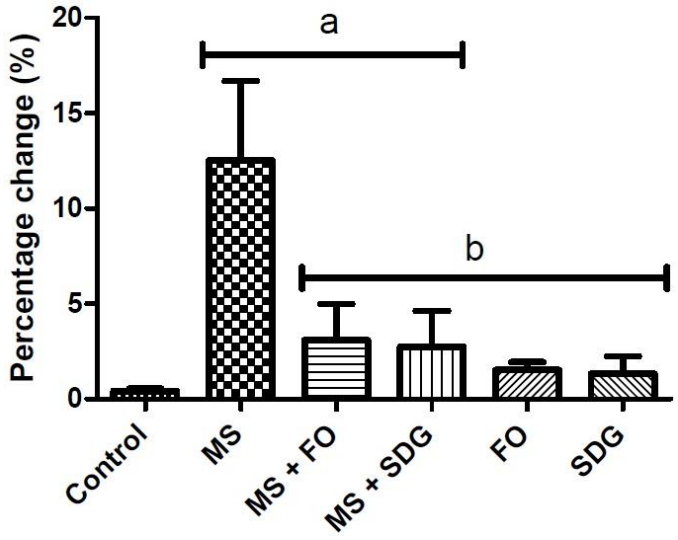


Figure 2

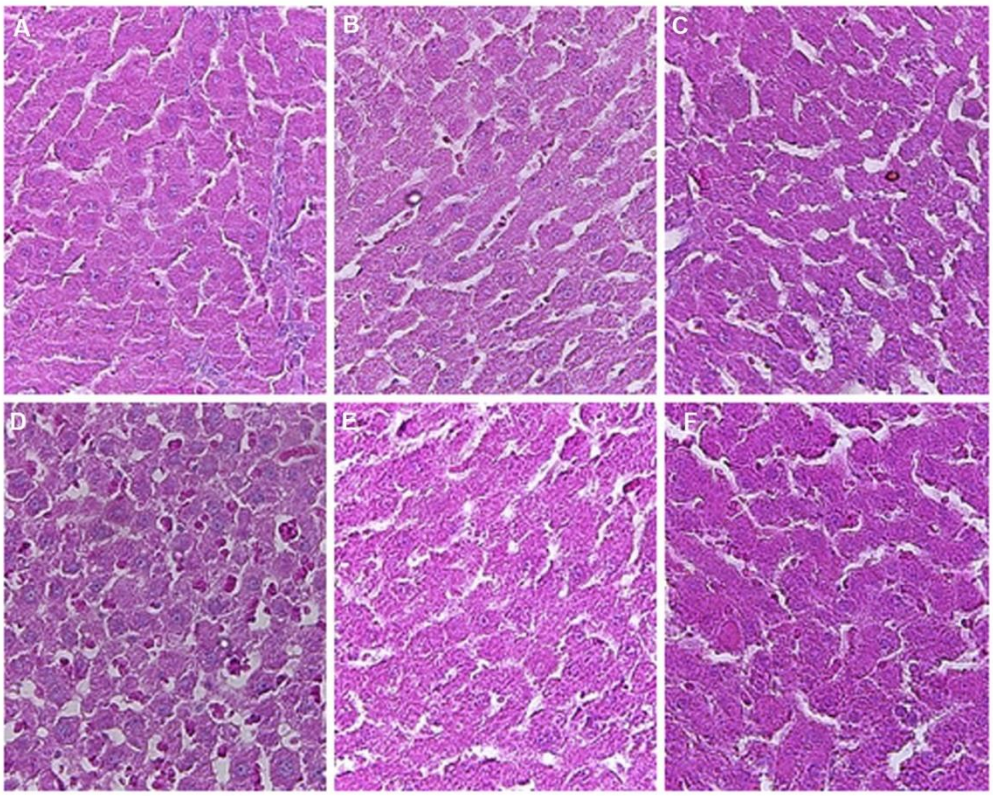
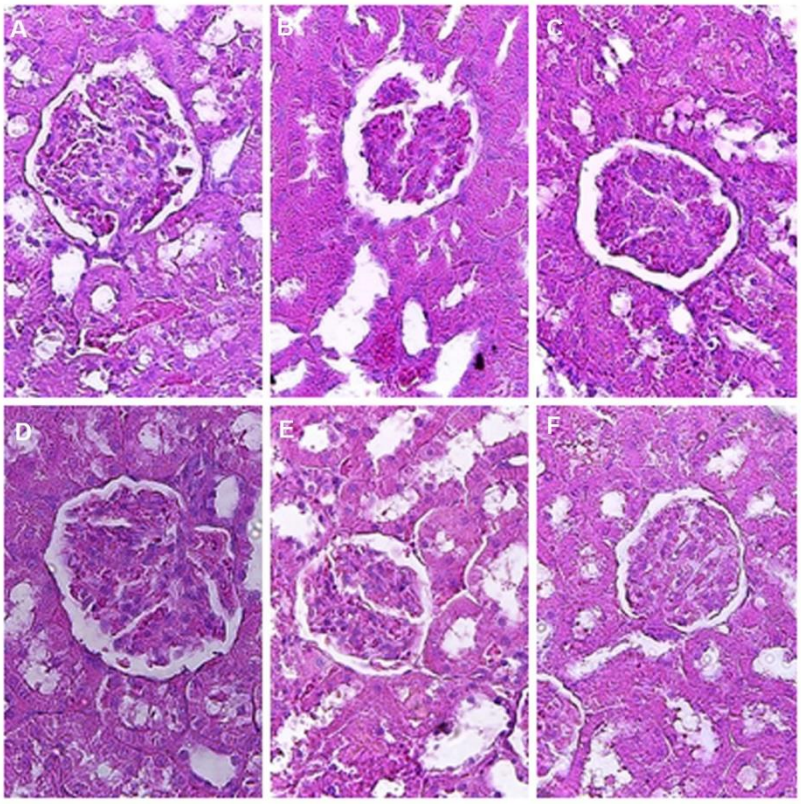


Figure 3



6 CONCLUSÃO

Os resultados deste trabalho indicam que:

- A suplementação com solução de frutose a 30% durante 30 dias é um modelo eficaz na indução de SM em ratos.
- 30 dias de suplementação com solução de frutose a 30% aumenta o estresse oxidativo, além de promover alterações inflamatórias e renais.
- O óleo de linhaça e a lignana SDG previnem o dano oxidativo a lipídios e proteínas, assim como a redução na atividade das enzimas anioxidantes e nos níveis de GSH provocados pela solução de frutose a 30%.
- O óleo de linhaça e a lignana SDG são capazes de prevenir o desenvolvimento de SM.
- O óleo de linhaça e a lignana SDG previnem alterações inflamatórias e renais associadas à SM.

Assim, sugere-se que tanto o óleo de linhaça quanto a lignana SDG isalada da semente de linhaça possam atuar na prevenção do desenvolvimento de SM, reduzindo, dessa forma, as complicações associadas a esse distúrbio.

REFERÊNCIAS

ABDEL-MONEIM, A. E.; DKHIL, M. A.; AL-QURAISHY, S. The potential role of flaxseed oil on lead acetate-induced kidney injure in adult male albino rats. **African Journal of Biotechnology,** v. 10, n. 8, p. 1436-1451, 2011.

ADLERCREUTZ, H. Lignans and Human Health. **Critical Reviews in Clinical Laboratory Sciences,** v. 44, n. 5-6, p. 483-525, 2007.

ALBERTI, K. G. M. M.; ZIMMET, P.; SHAW, J. The metabolic syndrome - a new worldwide definition. **Lancet,** v. 366, p. 1059, 1063, 2005.

ANDO, K.; FUJITA, T. Metabolic syndorme and oxidative stress. **Free Radical Biology and Medicine,** v. 47, n. 3, p. 213-218, 2009.

ANWAR, F.; PRZYBYLSKI, R. Effect of solvents extraction on total phenolics and antioxidant activity of extracts from flaxseed (*Linum usitatissimum* L.). **Acta Scientiarum Polonorum Technologia Alimentaria,** v. 11, n. 3, p. 293-301, 2012.

BARROS, C. M. et al. Substitution of drinking water by fructose solution induces hyperinsulinemia and hyperglycemia in hamsters. **Clinics**, v. 62, p. 327–334, 2007.

BERNACCHIA, R.; PRETI, R.; VINCI, G. Chemical Composition and Health Benefits of Flaxseed. **Austin Journal of Nutrition and Food Sciences,** v. 2, n. 8, p. 1045, 2014.

BLAND, J. S. Metabolic syndrome: the complex relationship of diet to conditions of disturbed metabolism. **Functional Foods in Health and Disease,** v. 2, p. 1-12, 2011.

BLOEDON, L. T. et al. Flaxseed and cardiovascular risk factors: Results from a double blind, randomized, controlled clinical trial. **Journal of the American College of Nutrition,** v. 27, n. 1, p. 65-74, 2008.

BOCARSLY, M. E. et al. High-fructose corn syrup causes characteristics of obesity in rats: increased body weight, body fat and triglyceride levels. **Pharmacology Biochemistry and Behavior,** v. 97, n. 1, p. 101-106, 2010.

BRASIL. Agência Nacional de Vigilância Sanitária. Resolução n.16 de 30 de abril de 1999. Regulamento técnico que estabelece as diretrizes básicas para análise e comprovação de

propriedades funcionais e ou de saúde alegadas em rotulagem de alimentos. **Diário Oficial [da] União,** Brasília, 1999.

BRASIL. Ministério da Saúde. **Alimentos regionais brasileiros.** 1. ed. Brasília: Ministério da Saúde, 2002.

CAMPOS, V. C.; TAPPY, L. Physiological handling of dietary fructose-containing sugars: implications for health. **International Journal of Obesity,** v.40, p. S6-S11, 2016.

COSTA, L. R. **Avaliação da composição mineral óssea em ratos wistar machos tratados com dieta contendo farinha de linhaça (*linum usitatissinum*) desde o período da lactação até os 180 dias de idade.** 2016. 59f. Trabalho de Conclusão de Curso – Faculdade de Nutrição Emília de Jesus Ferreiro – Universidade Federal Fluminense, 2016.

COUTO, A. N.; WICHMANN, F. M. A. Efeitos da farinha de linhaça no perfil lipídico e antropométrico. **Alimentos e Nutrição**, v. 22, n. 4, p. 601-608, 2011.

CUPERSMID, L. et al. Linhaça: composição química e efeitos biológicos. **E-Scientia,** v. 5, n. 2, p. 33-40, 2012.

ECKEL, R. H.; GRUNDY, S. M.; ZIMMET, P. Z. The metabolic syndrome. **Lancet,** v. 365, p. 1415-1428, 2005.

ECKEL, R. H. et al. The metabolic syndrome. **Lancet,** v. 375, n. 9710, p. 181-183, 2010.

EGERT, S. et al. Effects of an energy-restricted diet rich in plant-derived a-linolenic acid on systemic inflammation and endothelial function in overweight-to-obese patients with metabolic syndrome traits. **British Journal of Nutrition,** v. 112, p. 1315–1322, 2014.

FERNANDES, M. et al. Perfil de consumo de nutrientes antioxidantes em pacientes com síndrome metabólica. **Revista de Ciências Médicas,** v. 16, p. 209-219, 2007.

FLAX COUNCIL OF CANADA. Canada – a flax leader. Disponível em: http://flaxcouncil.ca/resources/about-flax/canada-a-flax-leader. Acessado em: 08/05/2017.

GLUBA, A. et al. Metabolic syndrome and renal disease. **International Journal of Cardiology,** v. 164, p. 141-150, 2012.

GOYAL, A. et al. Flax and flaxseed oil: an ancient medicine & modern functional food. **Journal of Food Science and Technology,** v. 51, n. 9, p. 1633-1653, 2014.

GRUNDY, S. M. et al. Definition of metabolic syndrome. **Circulation,** v. 109, p. 433-438, 2004.

GUIMARAES, P. S. Increasing angiotensin-(1–7) levels in the brain attenuates metabolic syndrome–related risks in fructose-fed rats. **Hypertension,** v. 63, p. 1078-1085, 2014.

GULATI, O. M.; OTTAWAY, P. B. Botanical nutraceuticals (food supplements, fortified and functional foods) in the European Union with main focus on nutrition and health claims. IN: BAGCHI, D. **Nutraceutical and Functional Food Regulations in the United States and Around the World.** New York: Academic Press, p. 199-219, 2008.

HALATENO, W. S. **Contaminação fúngica em sementes de linhaça comercializadas no município de Campo Mourão.** 2016. 38f. Trabalho de Conclusão de Curso – Curso Superior de Tecnologia de Alimentos – Universidade Federal Tecnológica do Paraná, 2016.

HERCHI, W. et al. A review of the methods used in the determination of flaxseed components. **African Journal of Biotechnology,** v.11, n. 4, p. 724-731, 2012.

HU, C; YUAN, Y. V.; KITTS, D. D. Antioxidant activities of the flaxseed lignan secoisolariciresinol diglucoside, its aglycone secoisolariciresinol and the mammalian lignans enterodiol and enterolactone in vitro. **Food and Chemical Toxicology**, v. 45, p. 2219–2227, 2007.

I-DBSM. SOCIEDADE BRASILEIRA DE CARDIOLOGIA / SOCIEDADE BRASILEIRA DE ENDOCRINOLOGIA E METABOLOGIA / SOCIEDADE BRASILEIRA DE DIABETES / ASSOCIAÇÃO BRASILEIRA PARA ESTUDOS DA OBESIDADE. I Diretriz Brasileira de Diagnóstico e Tratamento da Síndrome Metabólica. **Arquivos Brasileiros de Cardiologia,** v. 84, n. 1, p. 2-28, 2005.

IBGE. INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA. **Produção agrícola municipal: culturas temporárias e permanentes.** Rio de Janeiro: IBGE, v. 43, p.16, 2016.

IYER, A. et al. Potential health benefits of Indian spices in the symptoms of metabolic syndrome: a review. **Indian Journal of Biochemistry and Biophysics,** v. 46, p. 467-481, 2009.

KAJLA, P.; SHARMA, A., SOOD, D. R. Flaxseed – a potencial functional food source. **Journal of Food Science and Technology,** v. 52, n. 4, 1857-1871, 2015.

KASSI, E. et al. Metabolic syndrome: definitions and controversies. **BMC Medicine**, v. 9, P. 48-60, 2011.

KAUR, J. A comprehensive review on metabolic syndrome. Cardiology Research and Practice, v. 2014, p. 1-21, 2014.

KHALESI, S., JAMALUDDIN, R., ISMAIL, A. Effect of raw and heated flaxseed (*Linum usitatissimum* L.) on blood lipid profiles in rats. **International Journal of Applied Science and Technology**, v. 1, n. 4, p. 84-89, 2011.

KHAN, M. I. et al. Tackling metabolic syndrome by functional foods. **Reviews in Endocrine and Metabolic Disorders,** v. 14, p. 287-297, 2013.

KRAJA, A. T. et al. Pleiotropic genes for metabolic syndrome and inflammation. **Molecular Genetics and Metabolism,** v. 112, p. 317-338, 2014.

KYLIN, E. Studien. Hypertonie-hyperglykamie-hyperurikamiesyndrome. **Zentralblatt für innere Medizin,** v. 44, 1923.

LEIBOWITZ, A. et al. Role of T regulatory lymphocytes in the pathogenesis of high-fructose diet–induced metabolic syndrome. **Hypertension,** v. 61, p. 1316-1321, 2013.

LORENZO, C. et al. The metabolic syndrome as predictor of type 2 diabetes. **Diabetes Care,** v. 26, n. 11, p. 3153-3159, 2003.

MAMIKUTTY, N. et al. The establishment of metabolic syndrome model by induc|tion of fructose drinking water in male Wistar rats. **BioMed Research International,** v. 2014, p. 1-8, 2014.

MARQUES, A.C. **Propriedades funcionais da linhaça (*Linum usitatissimum* L.) em diferentes condições de preparo e de uso em alimentos.** 2008. 115 f. Dissertação – Mestrado em Ciência e Tecnologia de Alimentos – Centro de Ciências Rurais, Universidade Federal de Santa Maria, 2008.

MOLENA-FERNANDES, C. A. et al. Avaliação dos efeitos da suplementação com farinha de linhaça (*Linum usitatissimum* L.) marrom e dourada sobre o perfil lipídico e a evolução

ponderal em ratos Wistar. **Revista Brasileira de Plantas Medicinais,** v. 12, n. 2, p. 201-207, 2010.

MONEGO, M.A. **Goma da linhaça (*Linum usitatissimum* L.) para uso como hidrocolóide na indústria alimentícia**. 2009. 89 f. Dissertação – Mestrado em Ciência e Tecnologia de Alimentos – Centro de Ciências Rurais, Universidade Federal de Santa Maria, 2009.

MONIEM, A. E. A; DKHIL, M. A; AL-QURAISHY, S. Protective role of flaxseed oil against lead acetate induced oxidative stress in testes of adult rats. **African Journal of Biotechnology,** v. 9, n. 42, p. 7216-7223, 2010.

MORAES, F. P.; COLLA, L. M. Alimentos funcionais e nutracêuticos: definições, legislação e benefícios à saúde. **Revista Eletrônica de Farmácia,** v. 3, n. 2, p. 109-122, 2006.

NAGAO, K.; YANAGITA, T. Medium-chain fatty acids: Functional lipids for the prevention and treatment of the metabolic syndrome. **Pharmacological Research,** v. 61, p. 208-212, 2010.

NAKAGAWA, T. et al. A causal role for uric acid in fructose-induced metabolic syndrome. **American journal of physiology. Renal physiology**, v. 290, p. F625-F631, 2006.

NCEP-ATP III. The third report of the National Cholesterol Education Program (NCEP) Expert panel on detection, evaluation and treatment of high blood cholesterol in adults (Adult Treatment Panel III). **Journal of the American Medical Association,** v. 285, p. 2486-2497, 2001.

OLMEDILLA-ALONSO, B.; JIMÉNEZ-COLMENERO, F.; SÁNCHEZ-MUNIZ, F. J. Development and assessment of healthy properties of meat and meat products designed as functional foods. **Meat Science**, v. 95, p. 919-930, 2013.

OOMAH, B. D.; MAZZA, G.; KENASCHUK, E. O. Flavonoid content of flaxseed. Influence of cultivar and environment. **Euphytica,** v. 90, p. 163-167, 1996.

PANCHAL, S. K.; BROWN, L. Rodent Models for Metabolic Syndrome Research. **Journal of Biomedicine and Biotechnology,** v. 2011, p. 1-14, 2011.

PATEL, J.; IYER, A.; BROWN, L. Evaluation of the chronic complications of diabetes in a high fructose diet in rats. **Indian Journal of Biochemistry and Biophysics,** v. 46, n. 1, p. 66-72, 2009.

PATTYN, N. et al. The effect of exercise on the cardiovascular risk factors constituting the metabolic syndrome. **Sports Medicine**, v. 43, p. 121-133, 2013.

PILAR, B. C. et al. 28-days dietary supplementation with golden flaxseed improves biochemical and oxidative parameters in patients with metabolic syndrome. **Journal of Functional Foods,** v. 10, p. 232-242, 2014.

PORTELA, C. L. M. **Inter-relações entre síndrome metabólica, doença hepática gordurosa não alcoólica e fatores nutricionais em idosos hipertensos.** 2010. 141f. Dissertação – Mestrado em Saúde Pública – Universidade Estadual do Ceará, 2010.

POUDYAL, H. et al. Effects of ALA, EPA and DHA in high-carbohydrate, high-fat diet-induced metabolic syndrome in rats. **Journal of Nutritional Biochemistry**, v. 24, p. 1041–1052, 2013.

RAJESHA, J. et al. Antioxidant potentials of flaxseed by in vivo model. **Journal of Agricultural and Food Chemistry,** v. 54, n. 11, p. 3794-3799, 2006.

REAVEN, G. M. Banting lecture 1988. Role of insulin resistance in human disease. **Diabetes,** v. 37, p. 1595–1607, 1988.

REDLICH, R. **Estratégias de manejo de adubação na produção da linhaça (*Linum usitatissimum* L.).** 2016. 36f. Trabalho de Conclusão de Curso – Faculdade de Agronomia – Universidade Regional do Noroeste do Estado do Rio Grande do Sul, 2016.

RHEE, Y.; BRUNT, A. Flaxseed supplementation improved insulin resistance in obese glucose intolerant people: a randomized crossover design. **Nutrition Journal,** v. 10, p. 44-50, 2011.

RONCAL, C. A. et al. Combination of captopril and allopurinol retards fructose-induced metabolic syndrome. **American Journal of Nephrology,** v. 30, p. 399-404, 2009.

RUBILAR, M. et al. Flaxseed as a source of functional ingredientes. **Journal of Soil Science and Plant Nutrition,** v. 10, n. 3, p. 373-377, 2010.

SANTOS, C. R. B. et al. Fatores dietéticos na prevenção e tratamento de comorbidades associadas à síndrome metabólica. **Revista de Nutrição,** v. 19, n. 3, p. 389-401, 2006.

SCALBERT, A.; WILLIAMSON, G. Dietary intake and bioavailability of polyphenols. **The Journal of Nutrition,** v.130, p. 2073S-2085S, 2000.

SHAHIDI, F. Nutraceuticals and functional foods: whole *versus* processed foods. **Trends in Food Science & Technology,** v. 20, p. 376-387, 2009.

SILVA, L. C. **Concentração de ácidos graxos polinsaturados no leite de mulheres doadoras antes e após suplementação com linhaça ou ômega-3.** 2014. 75 f. Dissertação – Mestrado em Ciências da Saúde – Universidade Federal do Mato Grosso do Sul, 2014.

SIRÓ, I. et al. Functional food. Product development, marketing and consumer acceptance -a review. **Appetite,** v. 51, p. 456-467, 2008.

SMEDS, A. I. et al. Quantification of a broad spectrum of lignans in cereals, oilseeds, and nuts. **Journal of Agricultural and Food Chemistry,** v. 55, p. 1337-1346, 2007.

SOUZA, R. L. A. **Estudo da funcionalidade de espécies comestíveis do semiárido nordestino e estratégias para sua utilização como ingredientes para fins alimentícios.** 2014. 145 f. Tese – Doutorado em Engenharia Química – Universidade Federal do Rio Grande do Norte, 2014.

TAYLOR, C. G. et al. Dietary milled flaxseed and flaxseed oil improve N-3 fatty acid status and do not affect glycemic control in individuals with well-controlled type 2 diabetes. **Journal of the American College of Nutrition,** v. 29, n. 1, p. 72-80, 2010.

TRUCOM, C. **A importância da linhaça na saúde.** São Paulo: Alaúde Editorial, 2006.

VAGUE, J. La differenciation sexuelle, facteur determinant des formes de l'obesite. **La Presse Médicale,** v. 30, p. 339-340, 1947.

VI-DBH. SOCIEDADE BRASILEIRA DE CARDIOLOGIA / SOCIEDADE BRASILEIRA DE HIPERTENSÃO / SOCIEDADE BRASILEIRA DE NEFROLOGIA. VI Diretrizes Brasileiras de Hipertensão. **Arquivos Brasileiros de Cardiologia,** v. 9, n. 1, p. 1-51, 2010.

VIZZOTTO, M.; KROLOW, A. C.; TEIXEIRA, F. C**. Alimentos funcionais: conceitos básicos**. 1. ed. Pelotas: Embrapa Clima Temperado, 2010.

VOS, M. B.; LAVINE, J. E. Dietary fructose in nonalcoholic fatty liver disease. **Hepatology,** v. 57, p. 2525-2530, 2013.

WHO - World Health Organization. Obesity: preventing and managing the global epidemic, Report of a WHO consultation. **World Health Organization Technical Report Series,** v. 894, p. 1-253, 2000.

WU, H. et al. Lifestyle counseling and supplementation with flaxseed or walnuts influence the management of metabolic syndrome. **The Journal of Nutrition**, v. 140, p. 1937-1942, 2010.

ZAWISTOWSKI, J. Regulation of functional foods in selected Asian countries in the Pacific Rim. IN: BAGCHI, D. **Nutraceutical and Functional Food Regulations in the United States and Around the World.** New York: Academic Press, p. 365-401, 2008.

ZHANG, W. et al. Dietary flaxseed lignan extract lowers plasma cholesterol and glucose concentrations in hypercholesterolaemic subjects. **British Journal of Nutrition,** v. 99, p. 1301-1309, 2008.

ZURAVSKI L. et al. Protective Role of Golden Flaxseed (*Linum usitatissimum* L.) Against Oxidative Damage in Lipids and Proteins of Healthy Volunteers. **Journal of Biosciences and Medicines,** v. 3, p. 45-53, 2015.

ANEXO A - Parecer de aprovação do Comitê de Ética no Uso de Animais (CEUA) da UNIPAMPA

MINISTÉRIO DA EDUCAÇÃO
FUNDAÇÃO UNIVERSIDADE FEDERAL DO PAMPA
(Lei nº 11.640, de 11 de janeiro de 2008)

Pró-Reitoria de Pesquisa

COMISSÃO DE ÉTICA NO USO DE ANIMAIS - CEUA

Fone: (55) 3413 4321, E-mail: ceua@unipampa.edu.br

CERTIFICADO DE APROVAÇÃO DE PROTOCOLO PARA USO DE ANIMAIS EM PESQUISA

Número de protocolo da CEUA: **024/2014**

Título: **Avaliação dos efeitos do ácido α-linolênico e de um complexo de lignanas isolados da semente de Linum usitatissimum L. (linhaça) em ratos Wistar com Síndrome Metabólica**

Data da aprovação: **17/10/2014**

Período de vigência do projeto: De: **10/2014** Até: **10/2017**

Pesquisador: **VANUSA MANFREDINI**

Campus: **URUGUAIANA**

Telefone: **(55) 3402 0227**

E-mail: **vanusamanfredini@unipampa.edu.br**

Digitally signed by ALESSANDRA SAYURI KIKUCHI TAMAJUSUKU NEIS:98256009004
DN: cn=ALESSANDRA SAYURI KIKUCHI TAMAJUSUKU NEIS:98256009004, c=BR, o=ICP-Brasil, ou=RFB e-CPF A3, email=alessandratamajusuku@unipampa.edu.br

Professor Adjunto
Coordenadora da CEUA/UNIPAMPA