PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
MESTRADO EM CIÊNCIAS DA SAÚDE

LUDMILA PANTAROTO LIMA RIBEIRO

ANÁLISE DA *DYSPHANIA AMBROSIOIDES* COMO AGENTE PROTETOR DA PERDA DE MASSA ÓSSEA

Presidente Prudente - SP
2023

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Dissertação apresentada Pró-Reitoria de Pesquisa e Pós-Graduação, Universidade do Oeste Paulista, como parte dos requisitos para obtenção do título de mestre. - Área de concentração: Ciências da Saúde

Orientador:
Prof. Dr. Wilson Romero Nakagaki

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Presidente Prudente, 24 de março de 2023.

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DEDICATÓRIA

Esta pesquisa é dedicada a Deus, causa primordial de todas as coisas. Ao meu filho Joaquim, afim de incentivar a importância da educação em nossas vidas. Aos meus familiares que sempre me apoiaram durante esta jornada, e em especial ao meu marido Evandro que permaneceu ao meu ladoincentivando a buscar minha melhor versão. Essa conquista é para vocês.

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“Causa estranheza àqueles que não estão familiarizados com o amor dedicado aos animais e as plantas, causa estranheza àqueles que não entendem que na terra existem outras formas de vida.” (Maura Watan)

RESUMO

Análise da *Dysphania ambrosioides* como agente protetor da perda de massa óssea

Os glicocorticoides apresentam efeitos adversos que podem desencadear a perda de densidade mineral óssea. Há evidências que demonstram que a *Dysphania ambrosioides* pode prevenir a perda de massa óssea. O objetivo do trabalho foi analisar possíveis efeitos do consumo da *Dysphania ambrosioides* em ossos de ratas submetidas a indução de perda de densidade mineral óssea por uso de dexametasona durante 7 semanas. Foram utilizadas 40 ratas Wistar distribuídas em quatro grupos: grupo controle (CT), grupo dexametasona (Dexa), grupo dexametasona ambrosoides 25 mg/kg (Ambro1) e grupo dexametasona ambrosoides 500 mg/kg (Ambro2). Foram realizados análises qualitativas e quantitativas no extrato bruto hidroalcoólico de *Dysphania ambrosioides,* sendo identificado a presença de saponinas 0,618 µg/ml, flavonoides 2,174 µg/ml, taninos 63,44 µg/ml e alcaloides 0,107 µg/ml. A dexametasona foi aplicada duas vezes na semana, enquanto a *Dysphania ambrosioides* foi administrada diariamente. O controle de peso semanal apresentou perda ponderal nos grupos que receberam a dexametasona, com atenuação da perda em Ambro2. Após a eutanásia dos animais, foram realizadas análise das propriedades estruturais e materiais e análise de Raman dos fêmures. No ensaio mecânico o grupo CT apresentou maiores valores de força máxima comparado aos grupos Dexa e Ambro2, e foi similar estatisticamente com os três grupos tanto para a deformação absoluta quanto para a rigidez estrutural. O grupo Dexa foi menos resistente à aplicação de força em relação ao grupo Ambro1, o qual foi similar ao grupo CT. O CT apresentou maior valor para a tensão máxima em relação aos grupos Dexa e Ambro2, e similar em relação ao grupo Ambro1. O grupo Ambro1 suportou maior tensão comparado aos grupos Dexa e Ambro2. O CT e Ambro1 apresentaram maiores valores de módulo elástico, o grupo CT apresentou diferença em relação aos grupos Dexa e Ambro2 e o grupo Ambro1 demonstrou diferença quando comparado aos grupos Dexa e Ambro2. Na análise de Raman a razão 430/1270 não apresentou diferença estatistica entre os quatro grupos. A razão 960/1660 o grupo CT apresentou diferença entre os demais grupos, e o grupo Dexa apresentou diferença entre o Ambro2. A razão 1070/1660 foi observado diferença estatística entre o grupo CT e os demais grupos. Assim, foi possível observar que a *Dysphania ambrosioides* possui efeitos promissores relacionados ao aumento da resistência óssea mediante a indução de osteoporose por glicocorticoides.

Palavras-chave: Osso; Densidade óssea; Glicocorticoide; *Dysphania ambrosioides.*

ABSTRACT

Analysis of *Dysphania ambrosioides* as a protective agent of bonemass loss

Glucocorticoids have adverse effects that can trigger loss of bone mineral density. There is evidence that Dysphania ambrosioides can prevent bone loss. The aim of this study was to analyze possible effects of Dysphania ambrosioides consumption on bones of female rats submitted to induction of loss of bone mineral density by using dexamethasone for 7 weeks. Forty female Wistar rats were divided into four groups: control group (CT), dexamethasone group (Dexa), dexamethasone ambrosoides 25 mg/kg group (Ambro1) and dexamethasone ambrosoides 500 mg/kg (Ambro2) group. Qualitative and quantitative analyzes were performed on the crude hydroalcoholic extract of Dysphania ambrosioides, identifying the presence of saponins 0.618 µg/ml, flavonoids 2.174 µg/ml, tannins 63.44 µg/ml and alkaloids 0.107 µg/ml. Dexamethasone was applied twice a week, while Dysphania ambrosioides was administered daily. Weekly weight control showed weight loss in the groups that received dexamethasone, with loss attenuation in Ambro2. After the euthanasia of the animals, structural and material properties were analyzed and Raman analysis of the femurs. In the mechanical test, the CT group showed higher values of maximum force compared to the Dexa and Ambro2 groups, and was statistically similar with the three groups both for absolute strain and for structural stiffness. The Dexa group was less resistant to force application than the Ambro1 group, which was similar to the CT group. CT showed a higher value for maximum tension in relation to the Dexa and Ambro2 groups, and similar in relation to the Ambro1 group. The Ambro1 group withstood greater tension compared to the Dexa and Ambro2 groups. CT and Ambro1 showed higher elastic modulus values, the CT group showed a difference in relation to the Dexa and Ambro2 groups, and the Ambro1 group showed a difference when compared to the Dexa and Ambro2 groups. In the Raman analysis, the 430/1270 ratio showed no statistical difference between the four groups. The ratio 960/1660, the CT group showed a difference between the other groups, and the Dexa group showed a difference between the Ambro2. The 1070/1660 ratio showed a statistical difference between the CT group and the oand hus, it was possible to observe that Dysphania ambrosioides has promising effects related to increased bone strength through the induction of osteoporosis by glucocorticoids.

Keywords: Bone; Bone density; Glucocorticoid; *Dysphania ambrosioides.*

LISTA DE SIGLAS

Ambro1	– Grupo *Dexametasona Ambrosioides* 1
Ambro2	– Grupo *Dexametasona Ambrosioides* 2
CEUA	– Comissão de Ética no Uso de Animais
CH_2	– Hidrocarboneto
COBEA	– Colégio Brasileiro de Experimentação Animal
CSMI	– Momento de Inércia de Secção Transversal
CT	– Grupo Controle
Dexa	– Grupo Dexametasona
DMO	– Densidade Mineral Óssea
GC	– Glicocorticoides
LB	– Liberman - Burchard
OMS	– Organização Mundial da Saúde
RENISUS	– Relação Nacional de Plantas Medicinais de Interesse do SUS
UNICAMP	– Universidade Estadual de Campinas
UNOESTE	– Universidade do Oeste Paulista
V_1CO3^{2-}	– Carbonato
V_1PO4^{3-}	– Fosfato V1
V_2PO4^{3-}	– Fosfato V2
V_4PO4^{3-}	– Fosfato V4
vC-C	– Hidroxiprolina de colágeno

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ANEXOS

ANEXO A – Normas Revista *LIFE SCIENCES*

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INTRODUCTION

Life Sciences is an international journal publishing articles that emphasize the molecular, cellular, and functional basis of therapy. All articles are rigorously reviewed. The Journal favors publication of full-length papers where modern scientific technologies are used to explain molecular, cellular and physiological mechanisms. Articles that merely report observations are rarely accepted. Articles should be written at a level accessible to readers who are non-specialists in the topic of the article themselves, but who are interested in the research. The Journal welcomes reviews on topics of wide interest to investigators in thelife sciences. We particularly encourage submission of focused reviews containing high-quality artwork and mechanistic diagrams.

IMPORTANT INFORMATION

- Submission of a paper will be held to imply that the manuscript contains original unpublished work and is not being submitted for publication elsewhere.
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ANEXO B – Aprovação do Comitê de Ética

UNOESTE - Universidade do Oeste Paulista

PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO

PPG - Programa de Pesquisa de Pós-Graduação
PEIC - Programa Especial de Iniciação Científica

Parecer Final

Declaramos para os devidos fins que o Projeto de Pesquisa intitulado **"ANÁLISE DA DYSPHANIA AMBROSIOIDES COMO AGENTE PROTETOR DA PERDA DE MASSA ÓSSEA"**, cadastrado na Coordenadoria de Pesquisa, Desenvolvimento e Inovação (CPDI) sob o número nº **7249** e tendo como participante(s) **LUDMILA PANTAROTO LIMA RIBEIRO (discente), ELLEN FERNANDA TORRIANI DA SILVA (discente), TAIS MIRANDA DA CONCEICAO AMARO (discente), CARLOS JOSÉ LEOPOLDO CONSTANTINO (participante externo/voluntário), WILSON ROMERO NAKAGAKI (orientador responsável)**, foi avaliado e **APROVADO** pelo **COMITÊ ASSESSOR DE PESQUISA INSTITUCIONAL (CAPI) e COMISSÃO DE ÉTICA USO DE ANIMAIS (CEUA)** da Universidade do Oeste Paulista - UNOESTE de Presidente Prudente/SP.

Este Projeto de Pesquisa, que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica, encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de Outubro de 2008, do Decreto nº 6.899, de 15 de Julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), tendo sido APROVADO em reunião realizada em 11/02/2022.

Vigência do projeto: 05/2022 a 04/2024.

ANIMAL VIVO

Espécie/Linhagem/Raça	Nº de Animais	Peso	Idade	Sexo	Origem
Rattus novergicus (Wistar)	40	200 gramas	1 meses	F	Cemib / Unicamp

Presidente Prudente, 12 de Maio de 2022.

Prof. Dr. Jair Rodrigues Garcia Jr.
Docente Responsável pela CPDI

Prof. Dr. Felipe Rydygier de Ruediger
Coordenador da CEUA - UNOESTE

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