UNIVERSIDADE ESTADUAL DE MONTES CLAROS

Daniele Cristina Moreira

Avaliação do efeito homeopático de *Syzygium jambolanum* no tecido adiposo em camundongos no controle de diabetes mellitus tipo-2 induzido por dieta.

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Orientador: Prof. Dr. Sérgio Henrique Sousa Santos

Coorientador: Prof. Dr. Carlos Eduardo Mendes D'Angelis

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MESTRANDO(A): DANIELE CRISTINA MOREIRA

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RESUMO

A obesidade constitui um sério problema de saúde pública mundial, sendo caracterizado pelo excesso da gordura corporal. É um dos componentes chave do desenvolvimento da síndrome metabólica, que conduz várias complicações que impactam diretamente na expectativa e qualidade de vida. Complicações cirúrgicas e efeitos colaterais dos medicamentos contribuem para a busca de novas terapias. Dentre as alternativas encontra-se a homeopatia, prática milenar, no Brasil, que utiliza diluições de substâncias advindas de minerais, plantas e animais. Preparo homeopático de *Syzygium jambolanum* são utilizados no controle glicêmico, porém seu papel ainda não é bem investigado. O presente estudo foi conduzido para investigar os efeitos da administração oral de preparação homeopática de *Syzygium jambolanum* comparado ao uso de metformina na expressão de genes da adipogênese e o perfil glicêmico em camundongos alimentados com dieta rica em gorduras e com baixo teor de carboidratos, avaliando seu perfil metabólico. Foram um total de 48 camundongos, Swiss, machos tratados, mantidos em condição padrão no biotério da Universidade Estadual de Montes Claros, Brasil, de acordo com os princípios éticos, resolução 176/2018. A obesidade foi induzida por uma dieta rica em gordura e açúcar, enquanto os camundongos controle receberam dieta padrão por 12 semanas, após o qual os animais receberam por 30 dias uma preparação de 20 µl / 100g/peso do animal de *S. jambolanum* ou uma dose de 250 mg / kg. De Metformina. Foram coletados e congelados em gelo seco o fígado, músculo e tecidos adiposos. Foram avaliados parâmetros de composição corporal, bem como parâmetros bioquímicos. A quantificação da expressão de mRNA nas amostras de tecido adiposo epididimal foi realizado através da técnica de reação em cadeia da polimerase em tempo real (RT-PCR). Para análise dos dados do teste de sensibilidade insulínica e tolerância a glicose foi utilizada a análise de variância de duas vias (Two-way ANOVA). Os dados foram expressos como a média ± DP (desvio padrão). O nível de significância foi previamente estabelecido em $p< 0.05$. Resultados: Os camundongos tratados com *S. jambolanum* mostraram um ganho de peso reduzido e um tecido adiposo menor do que os ratos tratados com metformina, e ambos os grupos melhoraram a sensibilidade à insulina. Esses achados podem ser devidos à modulação da via da adipogênese (CEPB e PPAR) e à termogênese da UCP1, melhorando a sensibilidade à insulina, que pode influenciar diretamente o tecido adiposo, conforme corroborado por outros estudos. Conclusão: A administração homeopática da preparação de *Syzygium jambolanum* leva a menor peso corporal, glicemia, colesterol total e maior lipoproteína de alta densidade (HDL), validando seu uso seguro nas práticas clínicas.

Palavras-chave: Terapias alternativas. Diabetes Mellitus. Adipogênese.

ABSTRACT

Obesity is a serious public health problem worldwide, being characterized by excess body fat. It is one of the key components of the development of the metabolic syndrome, which leads to several complications that directly impact on life expectancy and quality of life. Surgical complications and side effects of medications contribute to the search for new therapies. Among the alternatives is homeopathy, an ancient practice in Brazil, which uses dilutions of substances from minerals, plants and animals. Homeopathic preparation of Syzygium jambolanum are used in glycemic control, but their role is not well investigated. The present study was conducted to investigate the effects of oral administration of homeopathic preparation of Syzygium jambolanum compared to the use of metformin in the expression of adipogenesis genes and the glycemic profile in mice fed a high-fat and low-carbohydrate diet, evaluating its metabolic profile. There were a total of 48 Swiss mice, treated males, kept in standard condition in the vivarium of the State University of Montes Claros, Brazil, according to ethical principles, resolution 176/2018. Obesity was induced by a diet high in fat and sugar, while the control mice received a standard diet for 8 weeks, after which the animals received a preparation of 20 µl / 100g / weight of the animal for 30 days. S. jambolanum or a dose of 250 mg / kg. Metformin The liver, muscle and adipose tissues were collected and frozen on dry ice. Body composition parameters were evaluated, as well as biochemical parameters. The quantification of mRNA expression in samples of epididymal adipose tissue was performed using the polymerase chain reaction technique in real time (RT-PCR). For analysis of the insulin sensitivity and glucose tolerance test data, two-way analysis of variance (Two-way ANOVA) was used. The data were expressed as the mean ± SD (standard deviation). The level of significance was previously established at $p < 0.05$. Results: Mice treated with S. jambolanum showed reduced weight gain and less adipose tissue than rats treated with metformin, and both groups improved insulin sensitivity. These findings may be due to modulation of the adipogenesis pathway (CEPB and PPAR) and to the thermogenesis of UCP1, improving insulin sensitivity, which can directly influence adipose tissue, as corroborated by other studies. Conclusion: Homeopathic administration of the Syzygium jambolanum preparation leads to lower body weight, blood glucose, total cholesterol and higher high-density lipoprotein (HDL), validating its safe use in clinical practices.

Keywords: Alternative therapies. Diabetes Mellitus. Adipogenesis.

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LISTA DE ABREVIATURAS E SIGLAS

DM2	Diabetes Mellitus tipo 2 (DM2)
IR	Resistência à insulina
OAD	Terapia antidiabética oral
AMPK	Proteína Quinase Ativada por Monofosfato de Adenosina
OMS	Organização mundial de saúde
TAB	Tecido adiposo branco
IL-1β	Interleucina 1 beta
IL-6	Interleucina 6
TNF-α	Fator de necrose tumoral alfa
MCP-1	Proteína quimioatrativa de monócitos
LDL	Lipoproteína de baixa densidade
HDL	Lipoproteína de alta densidade
TAM	Tecido adiposo marrom
UCP1	Proteína desacopladora 1
PNPIC	Política Nacional de Práticas Integrativas Complementares
PICs	Práticas Integrativas e Complementares
SUS	Sistema único de saúde
C/EBP-α	CCAAT/Enhancer Binding Protein
PPAR	Proliferator-Activated Receptor

SUMÁRIO

1 INTRODUÇÃO

1.1 Obesidade

A obesidade é um agravo de saúde pública que acomete muitas nações. Em todo o mundo o predomínio da obesidade vem aumentando ao longo dos anos, desde 1975, a obesidade quase triplicou [1]. Se o mesmo patamar continuar a tendência será que em 2030, 58% da população adulta no mundo estará entre sobrepeso ou obesidade [2].

O aumento do número de casos de obesidade contribui para uma grande atenção do sistema de saúde e financeiro em todo o mundo. A obesidade é um distúrbio sistêmico e fator de risco para outras doenças como diabetes mellitus tipo 2 (DM2), hipertensão arterial e doenças cardiovasculares. As taxas elevadas de obesidade são preocupantes, pois esta situação está relacionada à inflamação crônica de baixo grau, a danos no fígado, comorbidades posteriores, como intolerância à glicose. A resistência à insulina (IR) está muito relacionada ao aumento de peso [3].

Os resultados danosos à saúde da obesidade foram supostos como efeito da causa direta do aumento de tecido adiposo em uma série de sistemas biológicos, incluindo resistência à insulina, intolerância à glicose, aumento da pressão arterial, inflamação de baixo grau e dislipidemia [4].

Na obesidade, alterações nos fenótipos acontecem no tecido adiposo, logo após o processo de inflamação (5, 6). O tecido adiposo branco (TAB) é um órgão de muitos fatores capaz de mudar suas dimensões, tamanho e estado inflamatório, em resposta ao fator de nutrição (7,8).

Em relação à estrutura celular, o TAB modifica a composição extracelular, a vascularização, tamanho e a situação inflamatória das células imunes infiltradas, níveis aumentados de ácidos graxos livres circulantes, fatores pró-inflamatórios solúveis, fator de necrose tumoral alfa (TNF-α) e proteína quimioatrativa de monócitos (MCP- 1) ativação e infiltração de células imunes em tecidos específicos. O excesso de peso é relacionado à dislipidemia, partes pequenas e espessas de lipoproteína de baixa densidade (LDL), diminuição de partículas de lipoproteína de alta densidade (HDL) e de triglicérides no sangue (5, 9).

Nos mamíferos, além do TAB há o Tecido Adiposo Marrom (TAM) que é responsável tanto pelo estoque de nutrientes como lipídios quanto por distribuir energia em forma de calor em um método definido como termogênese sem tremores. Os adipócitos

marrons são definidos por uma estrutura de gotículas lipídicas, multiloculares, com número maior de mitocôndrias e estoque da proteína desacopladora 1 (UCP1) que se encontra na membrana mitocondrial interna da célula (10).

O estímulo de TAM está certamente correlacionado com a quantidade desse tecido, o seu estado de ativação e causas ambientais, tais como baixas temperaturas. Em seres humanos, a apresentação repetida ao frio provoca o aumento da atividade do TAM. Essa elevação na ação do TAM também tem sido bem correlacionado com aumentos induzidos pelo frio no gasto de energia pela termogênese (11-14).

O tecido TAM é um mediador crítico da saúde metabólica e colabora para a termogênese pelo desacoplamento da fosforilação oxidativa através da ação da Ucp1. Pesquisas atuais identificaram que o frio e a ação dos receptores β3-adrenérgicos provocam a formação de adipócitos bege semelhantes com células marrons no tecido adiposo branco. A termogênese provocada por adipócitos marrom e / ou bege melhora a condição de obesidade e DM2 relacionada à dieta rica em gordura. Dessa forma, a formação de adipócitos marrom e bege podem prevenir a obesidade nas doenças metabólicas, de maneira efetiva, juntamente a DM2 (15).

1.2 Diabetes Mellitus tipo 2

Diabetes mellitus é uma doença endócrina caracterizada por hiperglicemia persistente, resultando em complicações de longo prazo. A elevação da glicação das proteínas está relacionada a causa das complicações agudas e crônicas da doença metabólica (16).

O aumento da glicemia é influenciado por múltiplos fatores genéticos combinados com fatores ambientais e não fisiológicas e pode ser ainda caracterizado por um processo inflamatório crônico e que podem ser tratados por meio de ações comportamentais e econômicas efetivas (17,18).

O funcionamento do fígado é um fator preponderante na doença metabólica. A formação de glicose pelo fígado é complexa e moderada por ações indiretas; a insulina regula a lipólise do tecido adiposo, e os ácidos graxos livres controlam a produção de glicose no fígado. O adipócito é um provável local de resistência à insulina hepática. Além do mais, os rins exercem um papel na regulação da produção de glicose; a desnervação renal diminuem o efeito da absorção de gordura para causar resistência à insulina. A glicose em si é um relevante mediador do metabolismo no fígado; depois de entrar no fígado, a glicose é

fosforilada e pode ser transportada como lactato. Usando a ligação dinâmica glicose e lactato, conseguimos avaliar a efetividade da glicose em animais e humanos (19).

A prevalência de indivíduos que apresentam DM2 está crescendo muito em todo o mundo, com 439 milhões de casos estimados até 2030. Entre 2010 e 2030, ocorrerá um aumento de 69% no número de adultos com diabetes em países em desenvolvimento e aumento de 20% nos países desenvolvidos (20).

Esta tendência é refletida no Brasil, onde a prevalência de diabetes é um dos casos mais relevantes do DM2 em lugares em desenvolvimento, sendo que o país é o quarto maior número de pessoas com diabetes (21). Conforme a Federação Internacional de Diabetes são cerca de 22 milhões de pessoas em 2015 (22).

O aumento da prevalência de diabetes, dos fatores de risco modificáveis para a doença (obesidade, comportamento sedentário e alimentação), bem como as complicações graves que podem ser difíceis de prevenir e tratar, confirma que a prevenção é a opção relevante para diminuir o peso da condição clínica. Há estudos controlados randomizados que a diabetes tipo 2 pode ser prevenida ou adiada por meio de intervenções de alteração do estilo de vida que objetiva adequar a dieta, aumentar a atividade física e diminuir o peso em indivíduos com alto risco de desenvolver a doença (23). No tratamento convencional a Metformina é recomendada como a primeira escolha de medicamento antidiabético oral, se não contraindicada. É considerado eficaz, seguro e de baixo custo e pode diminuir o risco de eventos cardiovasculares e até o óbito (24).

Dessa maneira, a droga de primeira escolha para regulação dos níveis glicêmicos mais utilizados é a metformina. O mecanismo de ação dessa medicação ainda não foi descoberto, atualmente, um provável mecanismo pelo qual a metformina desempenha sua ação farmacológica é por meio da ativação da enzima proteína quinase ativada por adenosina monofosfato (AMPK) (25).

Ainda que a prática convencional se apresenta de forma segura e eficiente, o tratamento medicamentoso apenas pode não ser suficiente haja visto que altas dosagens e uso desregrado do mesmo pode provocar efeitos adversos com maior facilidade. Desse ponto de vista surgiu a necessidade de uma abordagem de práticas complementares que possibilite a terapêutica concomitante (26)**.**

1.3 Práticas Integrativas e Complementares

Nos últimos anos tem-se observado no país um crescimento considerável pela utilização de técnicas naturais de terapias alternativas complementares (TACs) para o controle de doenças e a restauração do equilíbrio do corpo humano (27).

O benefício pelas terapias alternativas tem aumentado no mundo, e não se determina apenas a uma classe social, áreas rurais ou países de baixo desenvolvimento. Conforme dados da Organização Mundial de Saúde (OMS), as terapias complementares estão crescendo tanto nos países desenvolvidos como nos países em desenvolvimento. De acordo com as informações dessa organização 80% da população dos países em desenvolvimento faz uso de práticas tradicionais. Uma vez que o aumento da procura pelo uso de terapias naturais na assistência à saúde deve-se a diversos fatores, como: o alto custo da assistência médica privada associado ao alto custo de medicamentos, as dificuldades dos atendimentos prestados pelos serviços públicos em geral, constatação de que as terapias complementares são tão eficazes quanto a terapêutica tradicional, e que, se usadas corretamente não provocam efeitos adversos perigosos ao indivíduo (28).

Com intuito de garantir a integralidade na assistência à saúde, o Ministério da Saúde criou no ano de 2006 a Política Nacional de Práticas Integrativas e Complementares (PNPIC) no Sistema Único de Saúde (SUS), cuja implementação engloba argumentações de natureza política, técnica, econômica, social e cultural. Essa política consiste à necessidade de se conhecer, apoiar, incorporar e implementar vivências que vêm sendo aplicadas na rede pública de muitos municípios e estados do Brasil, entre as quais destacam-se aquelas na área da Medicina Tradicional Chinesa: acupuntura, Homeopatia, Fitoterapia. Os profissionais que atuam com as Práticas Integrativas e Complementares (PICs), incentivam o indivíduo a alcançar seu bem estar e equilíbrio, porque entendem que o corpo, bem como a natureza, tem a possibilidade própria de buscar o bem-estar e dessa maneira auxiliar na qualidade de vida (29).

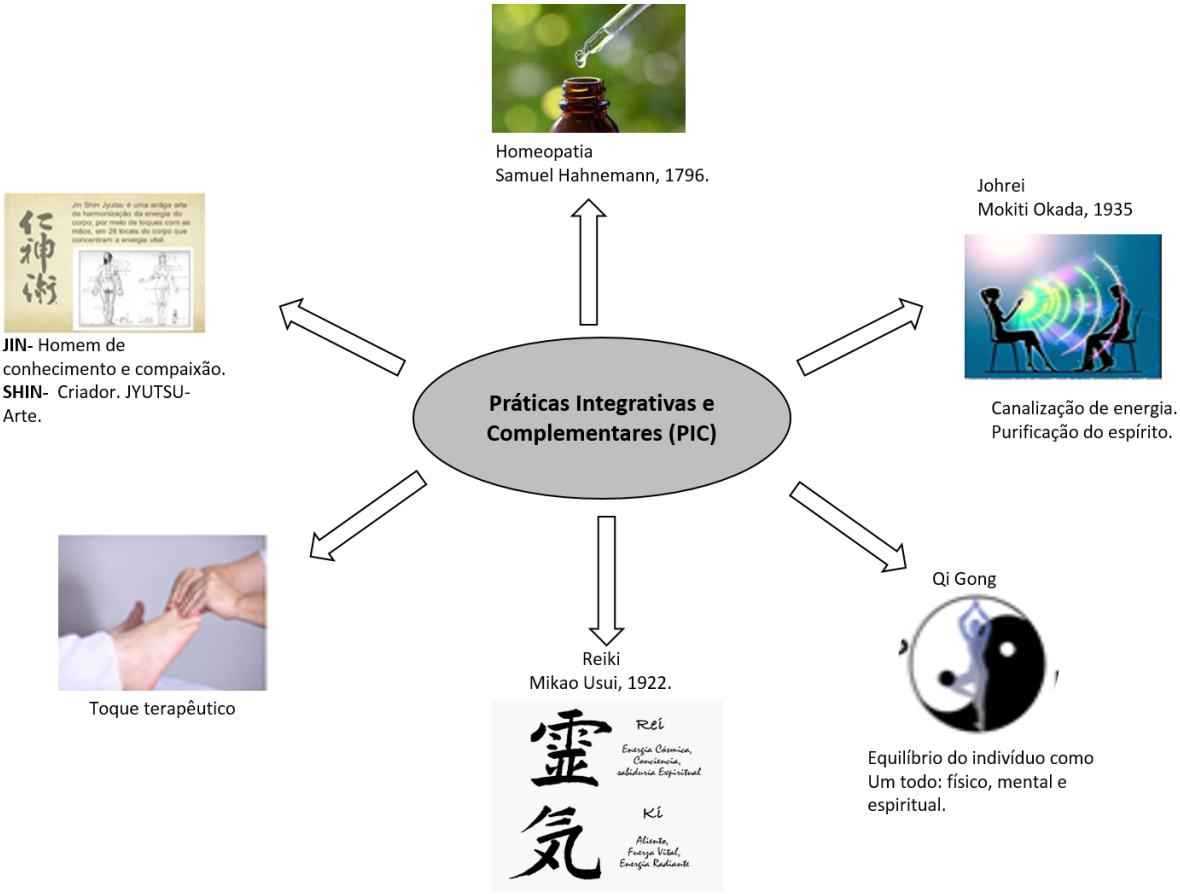


Figura1: Apresenta as terapias de práticas integrativas e complementares. Fonte: Imagens retirada do Google Imagens, 2018.

A população chinesa faz mais o uso de remédios fitoterápicos, os latinos optaram por uso de dietas e cura espiritual, os afros americanos têm como hábito usar a cura espiritual e os caucasianos costumam usar uma diversidade de técnicas que alternam entre físico, dietético, a massagem e acupuntura. Salientou-se que as divergências nas opiniões culturais têm um nível maior de impacto na procura por referências acerca da própria saúde do que só no nível de educação e na possibilidade de difundir a utilização das TACs para os trabalhadores de saúde (30).

O êxito, mas ainda as limitações dos tratamentos tradicionais incentivam a pesquisa em novas estratégias de terapêutica (31). Além das práticas tradicionais no tratamento de doenças, as alternativas crescem consideravelmente, a OMS propôs o aumento da regulamentação estatutária de praticantes e práticas de medicina tradicional e complementar, atualmente implementada em muitos países. Conforme os dados atuais da OMS, no entanto, a falta de diretrizes de políticas nesse meio retrata um impedimento significativo à elaboração de deliberações profissionais. (32). Como uma das terapias complementares (Figura 1) pode citar o toque terapêutico, reiki e a homeopatia, tornando disponíveis opções preventivas e terapêuticas aos pacientes.

1.4 Homeopatia

A homeopatia é um meio de tratamento que utiliza pequenas doses de substâncias naturais provenientes de plantas, minerais e animais (33). É um método medicinal que usa manipulações de substâncias cujos resultados quando administrados a pessoas saudáveis condizem às manifestações dos problemas de saúde, como sintomas, sinais clínicos, estados da doença no paciente de forma individualizada. O procedimento foi criado na Alemanha por Samuel Hahnemann (1755-1843) e atualmente é aplicado em todo o mundo (34).

É um dos sistemas de medicina complementar mais propagados com base nos dois princípios fundamentais "lei das semelhanças" e "diluição mínima. A homeopatia pode ser definida por medicamento, conhecido como alopatia por especialistas homeopáticos. A doutrina básica utilizada baseia em adotar o que se chama ``*similia smilibus curantur*`` ou a lei de similaridade, ou seja, os preparados têm a possibilidade de causar em um indivíduo saudável uma série de sintomas. Esta mesma substância usada em doses muito diluídas tem a capacidade para restaurar a saúde (35).

A particularidade em homeopatia, que requer adaptações métodos que não estão em acordo com as pesquisas e tratamentos tradicionais, consiste nos seguintes tópicos: a) o praticante deve levar em consideração o paciente global e individual condição e isso requer uma experiência muito ampla; b) o "remédio" é uma substância administrado em doses muito pequenas, em que os efeitos terapêuticos são previstos como experimentais em indivíduos saudáveis; c) métodos homeopáticos normalmente presume uma segunda receita baseada nas eficácias alcançadas após a primeira terapia; d) o efeito do tratamento homeopático deve ser analisado não levando em conta apenas o sintoma predominante que comumente leva o paciente a procurar o médico, mas também considerar a qualidade de vida do indivíduo (36).

O método de homeopatia como ação clínica busca a melhora da saúde por meio do reequilíbrio dinâmico e funcional do organismo doente e procura restabelecer as diferentes questões do indivíduo. Identificando o ser humano como um ser único que apresenta em seu organismo como doença apenas uma fase de um descontrole psíquico, emocional e de sua energia vital. O princípio homeopático leva em consideração o processo vital do paciente em seus fatores antropológico, psicofísico, funcional e terapêutico que pode ser essencial em seu restabelecimento geral para adesão de um estilo de vida saudável, equilibrado físico e mentalmente que são, na maioria das vezes, aspectos esquecidos na terapia preconizada pela metodologia médica tradicional (33).

A aplicação da terapêutica homeopática ao tratamento convencional foi relacionada a um melhor controle glicêmico em pacientes com DM2 em comparação com o tratamento convencional padrão isolado (37).

Dentre as preparações homeopáticas utilizadas no tratamento DM2, destaca-se nesta pesquisa a planta *Syzygium jambolanum* (SJ), pela ação hipoglicemiante já estudada, porém ainda é pouco esclarecido acerca do mecanismo (33).

1.5 *Syzygium jambolanum*

Para tratamento homeopático dentre as plantas mais utilizadas para o controle de diabetes mellitus tipo 2 o *Syzygium jambolanum* é uma das mais prescritas, porém seu papel ainda não é bem informado (33).

Figura 2: Imagem da árvore, folhas e frutos do SJ. Fonte: GUIMARÃES VHD, 2019.

O *Syzygium jambolanum* (Myrtaceae) é uma planta encontrada na Índia, no Paquistão, no sul da Ásia e no Brasil. A tintura mãe de *S. jambolanum* é amplamente utilizada por profissionais de homeopatia para o gerenciamento de diabetes. Tintura mãe é caracterizada como a tintura original que é feito com o uso de álcool, diretamente a partir da droga em estado bruto. É o precursor do preparo de diferentes potências e o ponto inicial para a fabricação de medicamentos diluídos (16,35).

Pertence às plantas medicinais mais regularmente utilizadas como terapia complementar no diabetes tipo 2 no mundo. O *S. jambolanum* foi muito estudada durante os

últimos 125 anos, aproximadamente 100 relatos de casos foram apresentados antes da descoberta da insulina. Depois da Segunda Guerra Mundial, a pesquisa foi concentrada em estudos com animais. Nem todos, porém muitos deles demonstraram algum êxito na diminuição dos sintomas do diabetes tipo 2 (38).

No Brasil, o S. *jambolanum* é uma das plantas medicinais mais utilizadas no tratamento do diabetes. As folhas, frutos e as cascas de S. *jambolanum* têm sido utilizadas por sua atividade hipoglicemiante. A adenosina desaminase (ADA) é uma enzima importante que exerce um papel relevante no metabolismo de purinas e DNA, respostas imunes e atividade de peptidase. Sugere-se que a ADA seja uma enzima importante para modular a bioatividade da insulina, mas seu significado clínico no diabetes mellitus (DM) ainda não foi comprovado. Sendo necessários estudos acerca do efeito de *S. jambolanum* sobre a atividade dos compostos em indivíduos hiperglicêmicos (39).

Pesquisa realizada com o uso do *S. jambolanum* em relação ao sistema cardiovascular demonstraou atividade hipotensora em ratos e vasorelaxação em anéis da artéria mesentérica, além disso, os resultados demonstraram que foi eficaz no tratamento de doenças cardiovasculares (40).

2 OBJETIVOS

2.1 Objetivo geral

Investigar os efeitos metabólicos da administração oral de preparação homeopática de *Syzygium jambolanum* e o uso de metformina em camundongos na expressão de genes da adipogênese e o perfil glicêmico em camundongos alimentados com dieta hipercalórica, avaliando seu perfil metabólico.

2.2 Objetivos específicos

2.2.1 Avaliar os efeitos do tratamento de animais diabéticos com uso de homeopatia e metformina na resistência insulínica.

2.2.2 Mensurar os níveis plasmáticos de biomarcadores do perfil lipídico, dosando especificamente os níveis de triglicérides, colesterol total e HDL.

2.2.3 Avaliar os testes de sensibilidade insulínica e tolerância a glicose, bem como avaliar os níveis glicêmicos.

2.2.4 Mensurar a expressão dos genes PPAR, CEBP e UCP1 na adipogênese e termogênese no tecido adiposo.

3 PRODUTO

3.1 Produto: *Syzygium jambolanum* homeopathic formulation improves diabetes modulating adipogenic genes in a diet-induced obese mice: comparison to the standard metformin treatment**.** Formatado segundo as normas para publicação do periódico: Complementary therapies in clinical practice.

3. PRODUTO

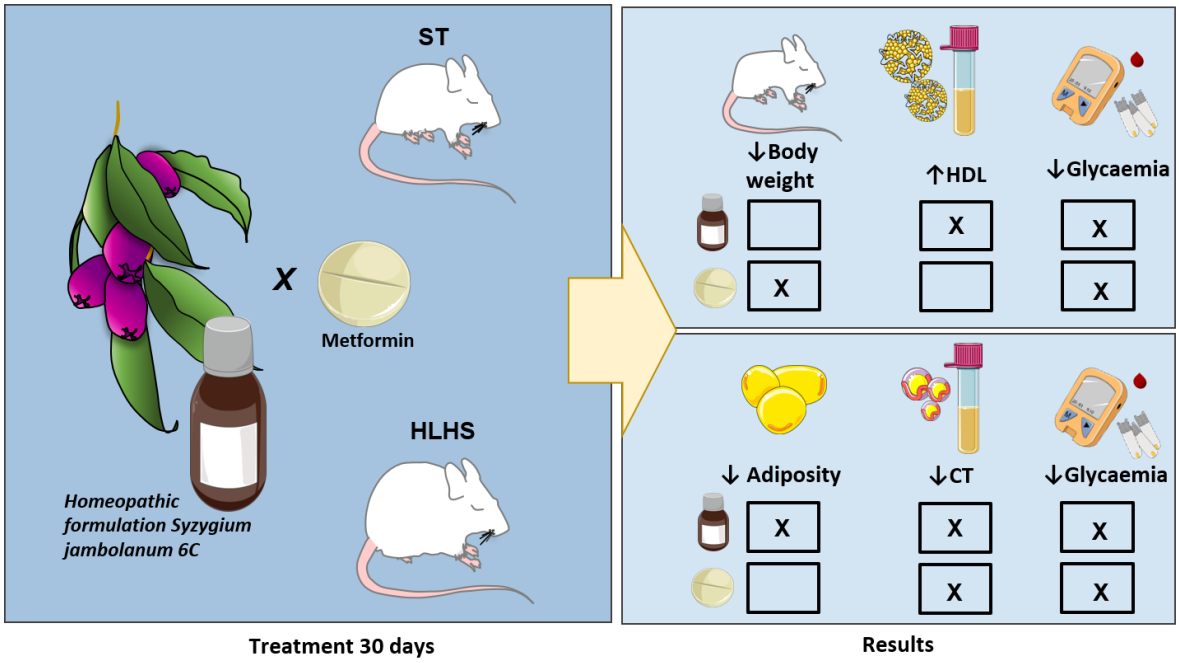
Syzygium jambolanum homeopathic formulation improves diabetes modulating adipogenic genes in a diet-induced obese mice: comparison to the standard metformin treatment

Short Title: _S. Jambolanum_ homeopathy improves diabetes in mice

ABSTRACT

Introduction: Homeopathy is an alternative therapy being a millenary practice that uses dilution. *Syzygium jambolanum* homeopathic preparation has a potential use to treat glycemic disorders. Aim: In this context, the aim of the present study was to evaluate the *Syzygium jambolanum* homeopathic preparation compared to metformin over diabetes and obesity in a mice model fed a high-fat/low-carb diet. Method: Male swiss mice were divided into six groups: obese and non-obese groups, treated and non-treated with *S. jambolanum* or metformin. Results: *S. jambolanum*-treated mice showed a reduced weight-gain and lower adipose tissue than metformin-treated mice, and both groups improved insulin sensitivity. These findings may be due to modulation of the adipogenesis pathway (CEPB and PPAR) and thermogenesis, of uncoupling protein 1 (UCP1). Conclusion: Homeopathic administration of *Syzygium jambolanum* preparation lead to lower body weight, blood glucose, total cholesterol and higher High-Densisity Lipoprotein (HDL), thus validating its safe use in clinical practices.

Graph abstract:



Key words: Alternative therapies. Diabetes Mellitus. Adipogenesis.

1. INTRODUCTION

Obesity is a multifactorial disturbance which, associated with overweight, represents over a third of world population. [1,2]. Should this trend be maintained, it is estimated that by 2030 over 58% of the adult population worldwide to be overweight or obese [3], such that this growing number of obesity cases presents a great challenge for both healthcare and finance systems worldwide

Obesity itself is a systemic deregulation and a risk factor for associated pathologies, such as Type 2 Diabetes (DM2), hypertension and cardiovascular diseases. One of the possible links between obesity and DM2 is insulin resistance (IR). Even though not all obese individuals present DM2 or insulin resistance (IR), there is, nevertheless, a high enough association [4].

IR contributes in many ways to Metabolic Syndrome, and one of the possible causes for IR is the elevated Non-Esterified Fatty acids (NEFAs), which are associated with increased adipose tissue mass, thus reducing glucose uptake and reducing adipocytes as well as myocytes insulin sensitivity, ultimately leading to DM2 development. [5].

According to World Health Organization (WHO), there are over 143 million patients suffering from diabetes, a number that is expected to grow 300 million by 2025. DM2 Conventional treatment includes lifestyle changes, such as nutritional habits, increased physical activities, anti-diabetic therapy and blood glucose evaluation [6]. Conventional DM2 therapy initial treatment is through use of metformin, a biguanide from the first choice oral hipoglicemiants class [7].

Associated with OMS 2014 and 2023 recommended traditional medicine, the use of Alternative and Complementary Therapy (ACT) is increasing in acceptance and popularity worldwide. The most common alternative treatment method for children with Diabetes in countries like Germany was homeopathy (14,5%) [8], while in Malaysia over half (56%) of diabetes patients used alternative therapies associated with traditional [9].

Homeopathy is one of such alternative practices, consisting on micro doses or ultra-dilutions of natural substances acquired from plants, minerals or animal parts [10], first practiced and implemented by German physician Samuel Hahnemann (1755-1843). It is based on the principle of the similar, "similia similibus curentur". Substances know to induce symptoms similar to those of a specific disease is offered to the patient in highly diluted

concentrations in order to enact self-regulation and self-healing processes [11]. In homeopathy, the medicine is chosen based on symptoms and overall aspect of the individual, [12] and the number of patients under homeopathic care quadrupled in a 7-year period during the 90s in the USA [13].

In homeopathy the successive dilution process is called potencialization, during which it is posited that the potentiated solution conserves the original substance characteristics accordingly to the rule of similar previously described [14].

Amongst homeopathic formulations for DM2 treatment is the plant *Syzygium jambolanum* (Jamun) in herb form [15,16]. Given that there are no researches on the possible pharmacological activity in the homeopathic treatment field, the molecular mechanisms underlying the homeopathic activity of *Syzygium jambolanum* remain unknown [17].

Therefore, the aim of the present study was to evaluate the oral administration effects of *Syzygium jambolanum* homeopathic preparation compared to metformin over adipogenesis-related genes, blood glucose profile and metabolic profile of mice fed a high-fat/low-carb diet.

The working hypothesis presented is that, compared to the traditional treatment, the addition of individualized homeopathic treatment for 30 days would lead to reduced blood glucose as well as other metabolic parameters resulting in an improved glycemic control.

2. MATERIAL E MÉTODOS

2.1 Animals

6-weeks old male Swiss mice (N=48) were kept in the Universidade Estadual de Montes Claros (UNIMONTES), MG bioterium under standard temperature [22 ± 2ºC], air humidity between 60 ± 5%, 12h light/dark cycle, low sound levels (under 40 dB) with balanced (50,3% de carbohydrates 41,9% de protein and 7,8% de fat 2,18 kcal/g Ração Presence®) chow diet and filtered tap water *ad libitum* [18]. Mice were kept in polypropylene boxes with 414 x 344 x 168 mm dimensions and galvanized steel lid.

Mice were separated in 6 groups of 8 individuals each accordingly to previous studies. Groups distribution can be observed in TABLE 1:

Table 1 – Experimental Groups

Group Description	Group ID
Standard Diet (ST).	**G1**
ST + Metformin (300 mg/Kg daily).	**G2**
ST+ *Syzygium jambolanum* (Sj-6c-20µl / 100g body weight of the animal) for 30 days [17].	**G3**
High-lard/high-sugar (HLHS).	**G4**
HLHS + Metformina (300mg/Kg daily).	**G5**
HLHS + *Syzygium jambolanum* (Sj-6c-20µl / 100g body weight of the animal) for 30 days [17].	**G6**

Treatment went on for 30 days and was masked so that the analysis researcher had no participation in the study up to that point, as to not skew the results accordingly to possible confirmation bias.

2.2 DM2 induction

High-Lard/High-Sugar (HLHS) rich diet for 90 days with the composition observed in TABLE 2. The diets composition were formulated according to Tatiane and cols [19], where the macronutrients, mineral and vitamins needs were supplied according to the American Institute of Nutrition [20].

Table 2- HLHS diet composition

Ingredients	**HLS** g/kg of Diet
Butylated hydroxytoluene	0.014
Choline bitartrate	2.5
Methionine	3
Vit. AIN93M	10
Minerals AIN93M	35
Cellulose	50
Starch	208.6
Casein	200
Soy oil	70
Lard	189
Açúcar	232

Composition (%)	
Carbohydrate	36.59
Proteins	12.88
Lipíds	50.53
Kcal/g	5.1

Source: All ingredients purchased from Rhoster® LTDA (São Paulo, SP, Brazil).

2.4 Homeopathic an Metformin Administration

Both *S. Jambolanum* and metformin were administered daily through gavage. *S. jambolanum* formulation acquired in a pharmacy specialized in homeopathy with centesimal redilution 6 (6C) accordingly to Hahnemann technique [17], 300 mg/kg metformin capsules were diluted in tap water and prepared at the time of administration by full body weight.

2.5 Food Intake

Food intake was evaluated twice a week on a semi-analytical scale subtracting the value obtained 24h after previous measure. Ingested food intake was corrected by mice total body weight. Mean for the values obtained was registered in researcher's field journal.

2.6 Body weight

Mice total body weight was evaluated twice a week always at the same time. Adipose tissues were evaluated after the animals were killed by decapitation by weighting epididymal, retroperitoneal, mesenteric and brown adipose tissues as well as gastrocnemius muscle and liver sample. All these tissues were removed, weighted, immediately frozen in liquid nitrogen and subsequently kept under -80º C.

2.7 Glucose Tolerance test and Insulin Sensitivity test

Glucose Tolerance Test (GTT) was performed by injecting mice fasted for 12h with 2 g/kg body weight of glucose. Blood glucose level was evaluated from samples obtained from the tail at 0, 15, 30, 60 e 120 min. Insulin Sensitivity test was performed by injecting non-fasted mice 0,75UI/kg body weight of insulin. Blood samples were collected at 0, 15, 30 e 60 minutes after insulin administration. Blod glucose was evaluated using Glucometer Accu-Check (Roche Diagnostics®, Indianapolis, EUA) [21].

2.8 Blood Sample

Blood was collected from 12h fasted mice killed by guillotine decapitation after treatment period. All samples had serum separated by 3200 rpm and 10 minutes centrifugation and kept in -80ºC. Serum total cholesterol, Triglycerides and glucose were evaluated using enzymatic kits (Wiener® Argentina).

2.9 Plasma levels of Total Cholesterol, HDL and triglycerides

Plasma levels of Total Cholesterol, HDL and triglycerides evaluated by de cholesterol oxidase method [22], using commercial kit.

2.10 Adipose Tissue Histology

Adipose tissue samples were kept in formaldehyde before transference to a 70% ethylic alcohol solution for subsequent paraffin inclusion. 7µm thick cuts were obtained from specific microtome followed by assembly in glass slides previously prepared and HE treated for adipocytes size analysis. The size was assessed on Carl Zeiss Axioskop 40 optical microscope (Gottingen, Germany) coupled to an AxioCam MRc (Carl Zeiss Imaging Systems) digital camera connected to a computer [5].

2.11 mRNA levels by Real-Time RT-PCR

Epididymal adipose tissue samples were treated with Trizol (Invitrogen Corp.VR, San Diego, CA, EUA) and DNAse (Invitrogen Corp.VR). Reverse transcription was performed with MMLV (Invitrogen Corp.VR) using random primers. mRNA levels of genes of interest (Table 3) were determined by Real-Time RT-PCR (SYBR GREEN Reagent) in Applied Biosystems® QuantStudio™ 6 Flex Real-Time PCR System equipment. Gene expression quantified using CT comparative relative method (Cycle limiar) using GAPDH as endogenous control [23].

Table 3 Sequence of primers used for real-time PCR analysis.

Gene	Primer sequence
C/EBP-α	Forward: 5. GTC TGC ACG TCT ATG CTA ACC CA-3 Reverse: 5. GCC GTT AGT GAA GAG TCT CAG TTT G-3
PPAR	Forward: 5. TTC AGC TCT GGG ATG ACC TT. 3 Reverse: 5. CGA AGT TGG TGG GCC AGA AT. 3
UCP1	Forward: 5. ACT GCC ACA CCT CCA GTC ATT. 3 Reverse: 5. CCT TGC CTC ACT CAG GAT TGG. 3
GAPDH	Forward: 5. GGG TGT GAA CCA CGA GAA AT-3 Reverse: 5. CCT TCC ACA ATG CCA AAG TT-3

C/EBP-α: CCAAT/Enhancer Binding Protein, PPAR: Peroxisome Proliferator-Activated Receptor, UCP1: Uncoupling-Protein 1 e GAPDH: Glyceraldehyde 3-phosphate dehydrogenase.

2.12 Statistical Analysis

Data analyzed using Graph Pad Prism 5.0 ®, (San Diego, EUA) e submitted to specific tests and statistical confidence of 95% ($p < 0,05$). Data expressed as Mean média ± SEM. Statistical differences between groups was evaluated by one-way ANOVO or two-way ANOVA followed by Tukey post-test.

2.13 Ethical Guidelines

The research followed national and international guidelines regarding animal research, submitted and approved by Comitê de Ética em Experimentação e Bem-Estar Animal (CEEBEA) under number 176 from Universidade Estadual de Montes Claros.

3. RESULTS

3.1. *Syzygium jambolanum*, diet and body composition

Epididymal adipose tissue was lower in HLHS + SJ group (0,0009123 ± 0,00009761) compared to HLHS group ($p < 0,008$); Total adiposity was also lower in HLHS + Sj group (0,01783 ± 0,002490), $p < 0,02$ compared to HLHS group. As noted, body weight gain was

lower in the ST control groups plus Sj (40.65 ± 0.4992) p <0.0008, when compared to the obese control group HLS plus Metformin (50.12 ± 0.96), p <0.01. (Figure 1)

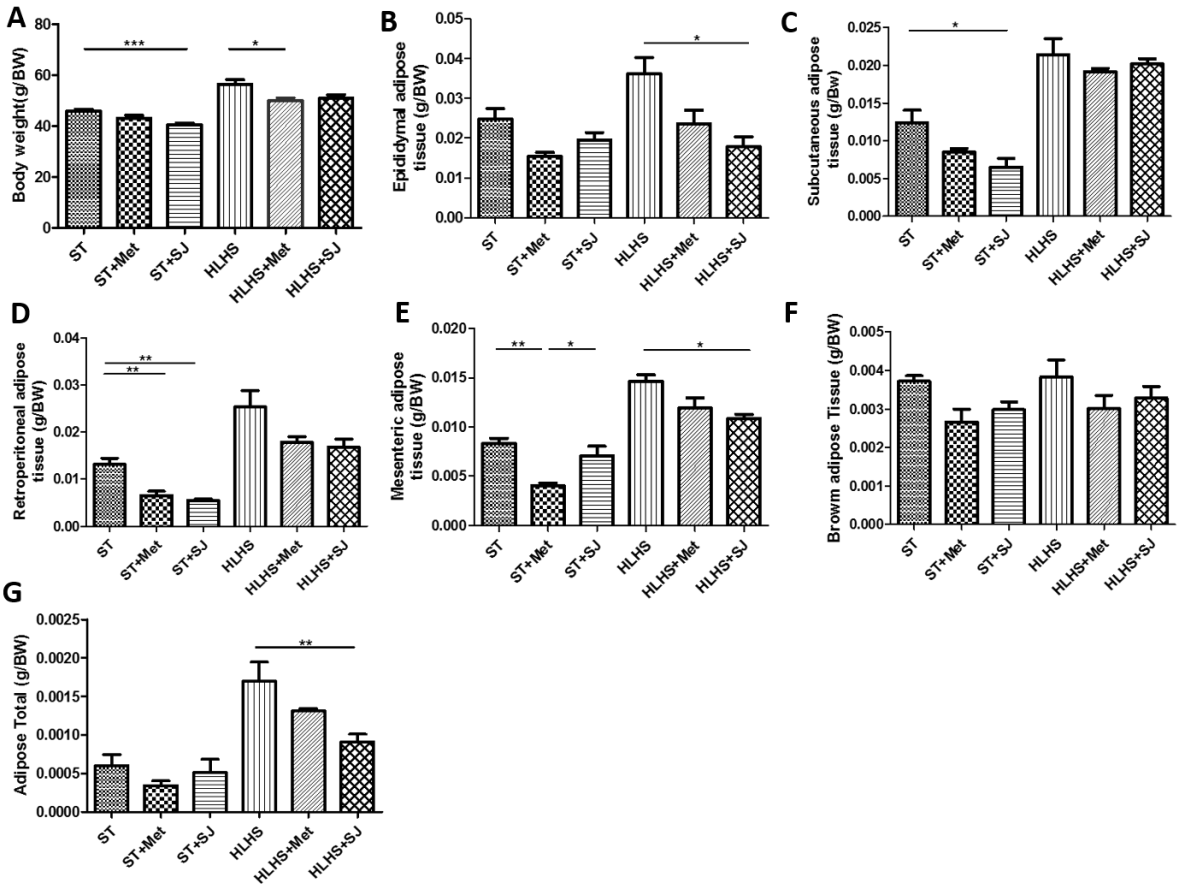


Figure 1: A) total body weight; B) epydydimal adipose tissue weight; C) suncutaneous adipose tissue weight; D) retroperitoneal adipose tissue weight; E) Mesenteric adipose tissue weight ; F) Brown adipose tissue weight; G) Total fat weight. BW: body weight; ST: standard diet, ST+ Met: ST plus Metformin, ST +SJ: ST plus *Syzygium jambolanum*; HLHS: high-lard/high-sugar, HLHS + Met: HLHS plus Metformin, HLHS+ SJ: HLHS plus *Syzygium jambolanum.*

3.2 Glycemic and biochemical parameters

It as observed and increased insulin sensitivity in ST + SJ (p <0,04) mice compared to ST mice (Fig. 2A) and an improved glucose tolerance (Fig. 2B).

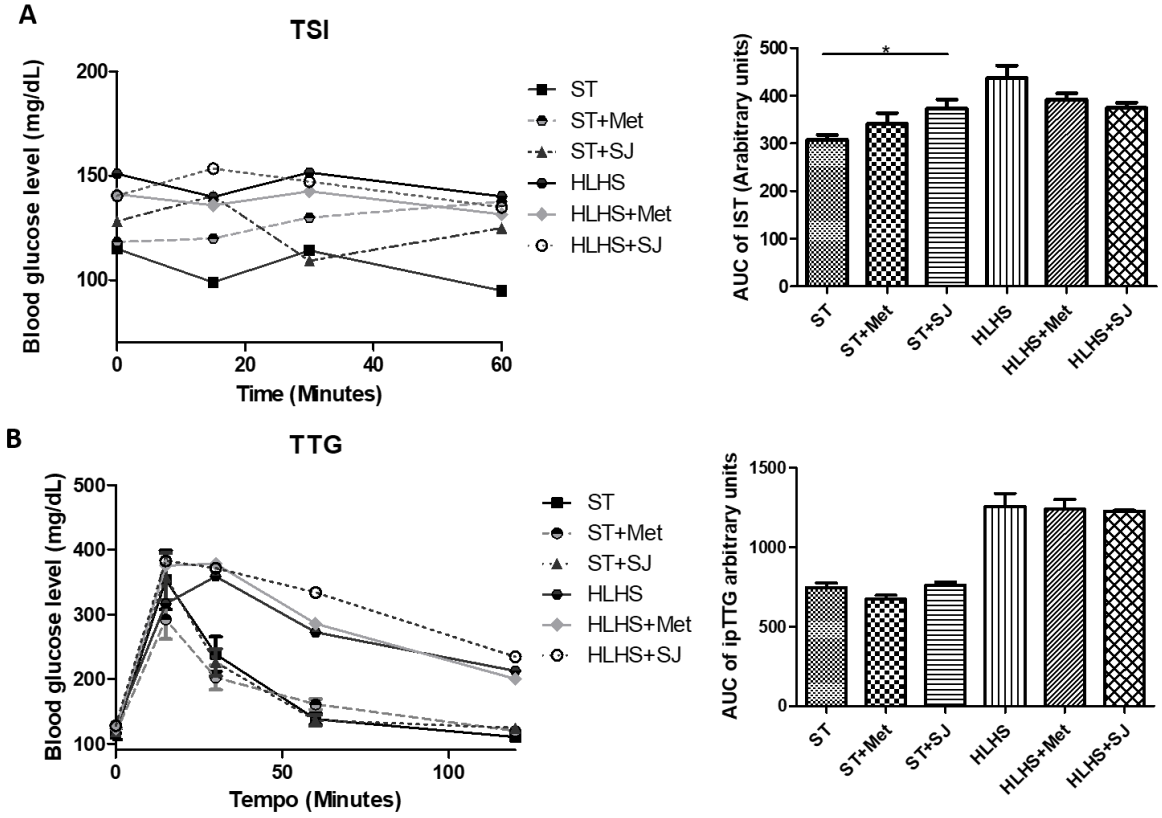


Figure 2: *Syzygium jambolanum* homephatic preparation effects over insulin sensitivity and glucose tolerance levels and Area Under Curve (AUC) (mg/dl) in mice fed a high fat/high carb diet. (A, B). ST: standard, ST + Met: ST + Metformin, ST + SJ: ST + *Syzygium jambolanum*. HLHS: high fat/high carb, HLHS + MET: HLHS + metformin, HLHS + SJ: HLHS + *S. jambolanum.*

It was also evaluated *S. jambolanum* and metformin effects over fasted swiss mice plasma glucose levels 0, 10 and 30 days after treatment, and it was found a reduced blood glucose in obese mice treated with either *S. jambolanum* or metformin (Figure.3).

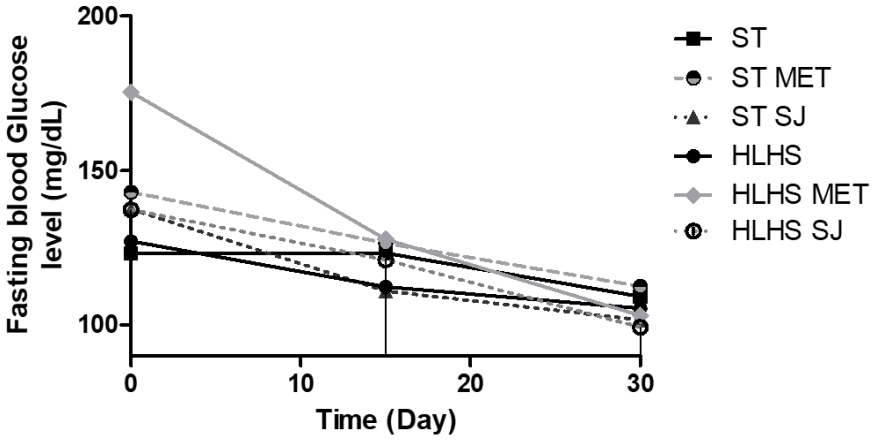


Figure 3: *Syzygium jambolanum* e metformina effects over fasted mice blood glucose levels in the first, tenth and thirtieth days. o dia. *p < 0.05. ST: standard, ST+Met: ST plus metformin, ST+SJ: ST plus *Syzygium jambolanum.* HLHS: High-lard/high-sugar, HLHS + MET: HLHS plus metformin, HLHS +SJ: HLHS plus *S. jambolanum*.

Blood glucose levels were different in entre ST mais Sj group (92,83 ± 2,750) p < 0,01 compared to ST group. Obese mice treated with HLHS + Sj showed lower blood glucose (90,67 ± 1,706) than obese mice treated only with Metformin (95,17 ± 2,428) p <0,04. Total cholesterol levels were also different in obese mice treated with SJ compared to mice treated only with metformin (105,7 ± 8,620 vs 112,0 ± 14,73) p < 0,001. HDL was also higher in non-obese mice treated o nly with SJ compared to non-obese mice treated only with metformin (141,8 ± 7,685 vs 107,5 ± 4,185) p < 0,007. (Figure 2). No differences were observed in creatinin, alkaline fosfatase, TGO, TGP and albumin between the groups analyzed (Figure 4).

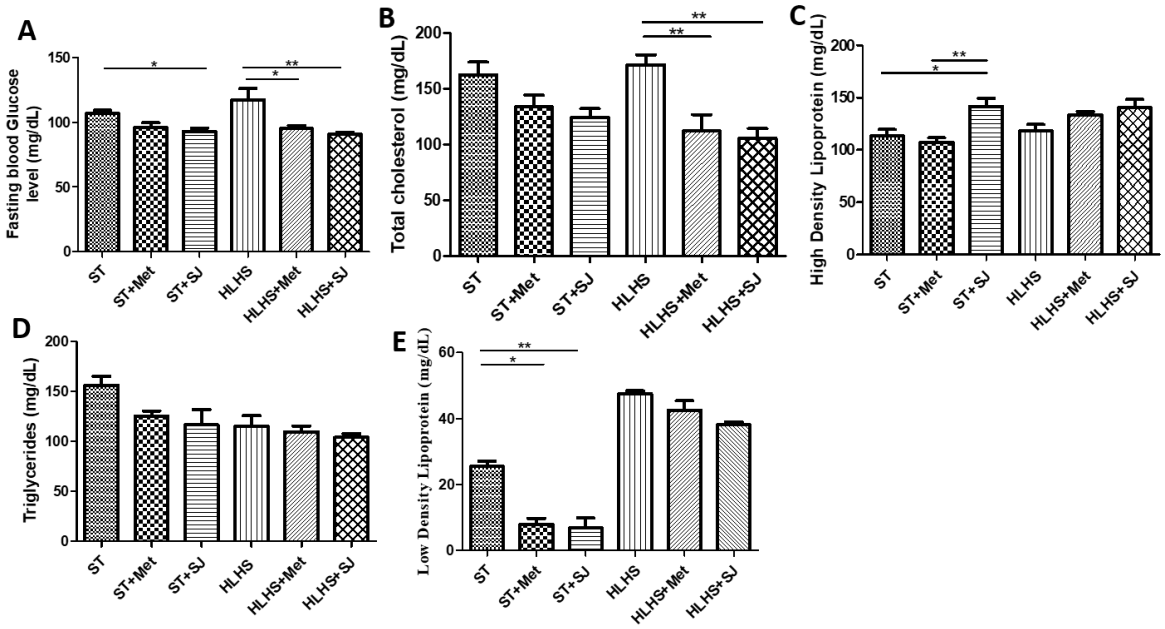


Figure 4: Fasting blood Glucose level (mg/dL) (A) and total cholesterol (CT) (mg/dL) (B). High Density Lipoprotein (mg/dL) (C) and Triglycerides (mg/dL) (D) and Low Density Lipoprotein (mg/dL) (E). *p < 0.05. ST: standard, ST+Met: ST plus metformin, ST+SJ: ST plus *Syzygium jambolanum.* HLHS: High-lard/high-sugar, HLHS + MET: HLHS plus metformin, HLHS +SJ: HLHS plus *S. jambolanum.*

3.3 Syzygium jambolanum and metformin adipogenic gene expression.

PPAR showed an increased expression in HLHS + SJ (1,02 ± 0,09) p<0,02, while CCAAT/Enhancer Binding Protein (CEPB alfa) was significantly reduced in ST+Met (0,45 ± 0,098) p<0,04 e ST +SJ (0,17 ± 0,11) p<0,006 and UCP1 showed and increased expression in HLHS+SJ group (0,19 ± 0,01) p<0,03 compared to obese control (Figure 5).

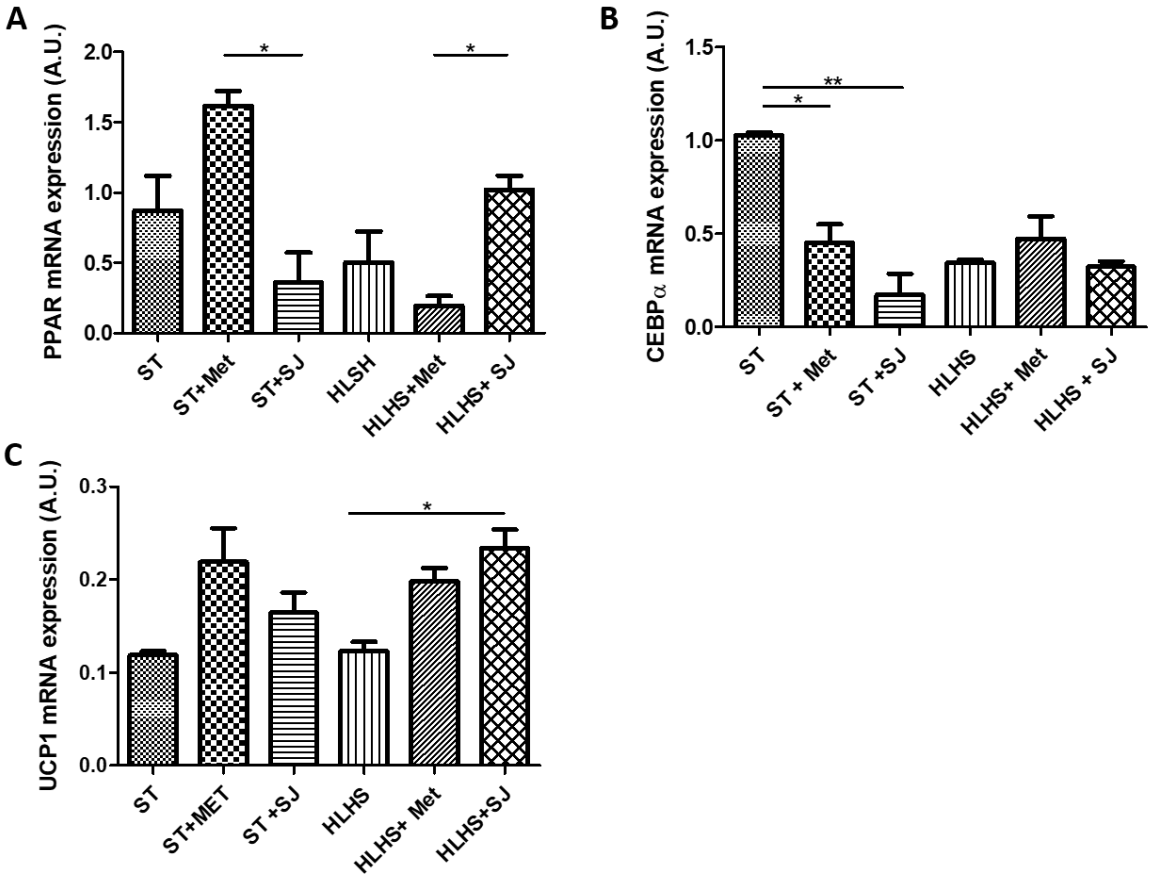


Figure 5: mRNA levels in epididymal adipose tissue in mice A) PPAR: Peroxisome proliferator-activated receptor, B) C/EBPα: CCAAT/Enhancer Binding Protein, C) UCP1: Uncoupling-Protein 1. * $p < 0,05$.

3.4 Histology

Adipocytes area was reduced in ST + SJ compared to the St + Met groups (1329000 ± 39260 vs 1983000 ± 79580 μm2). Moreover, both HLHS + Met (3365000 ± 161700 μm2) $p< 0,001$ and HLHS + SJ (3050000 ± 148300 μm2) $p< 0,005$ groups showed reduced adipocytes area compared to HLHS group (Figure 6).

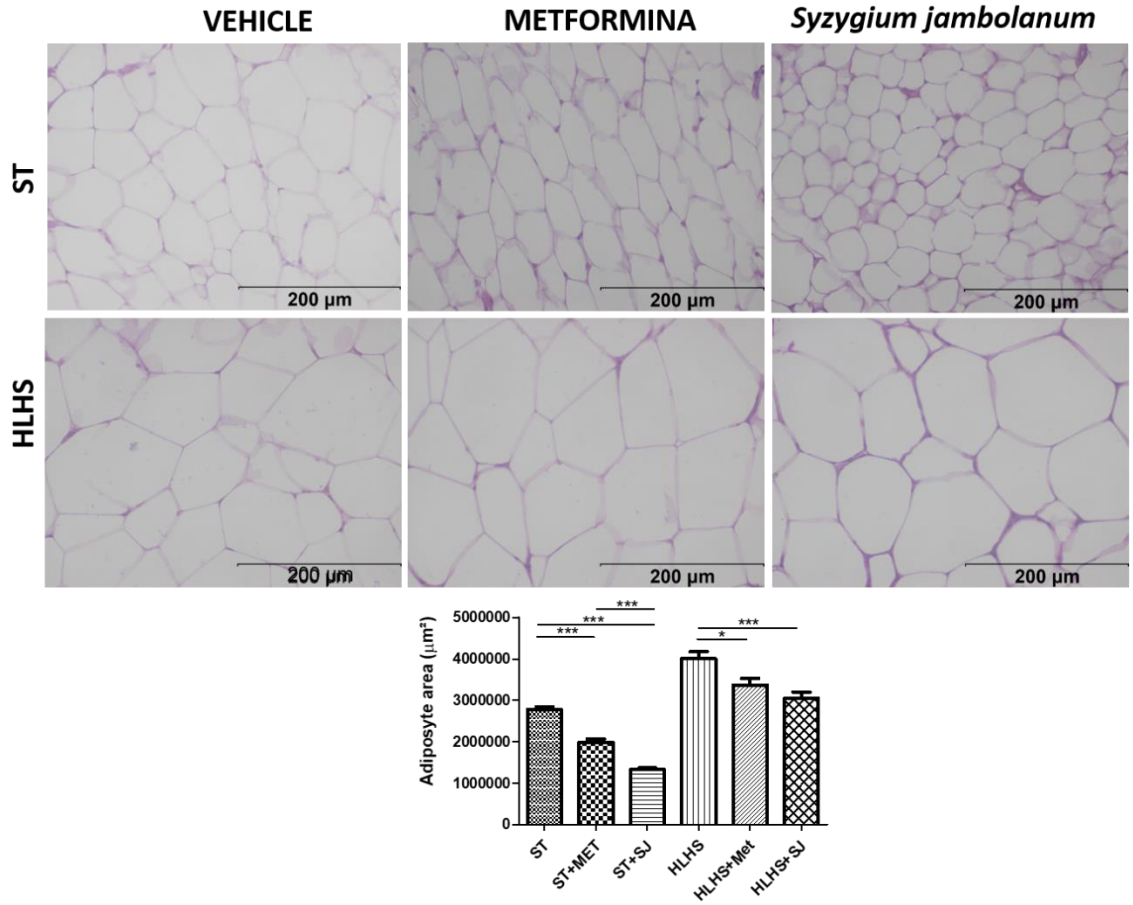


Figure 6: Histological analysis in a standard mouse-fed diet (ST), a diet high in fat and sugar (HLHS), HLHS plus metformin (HLHS + MET), HLHS + S. jambolanum (HLHS + SJ). Sections of tissue stained with hematoxylin-eosin (HE) of the epididymal adipose tissue and adipocyte area (µm2)). * p <0.05.

4. DISCUSSION

The main results of the present study showed that *S. jambolanum* homeopathic preparation may be an alternative therapy to treat obesity. Diet-induced obese mice treated with *S. jambolanum* (compared to metformin) showed reduced adiposity, body weight, blood glucose plasma levels, total cholesterol and increased HDL. The homeopathic preparation also modulated gene expression related to adipogenesis and thermogenesis in adipose tissue.

S. jambolanum phytochemistry compounds and allopathic effects has being described over diabetes and obesity treatment and are likely connected to the interaction between all the chemical structures present in the preparation, such as flavonoids, galic acid, ellagic acid,

glicosides, triteripenoids and saponins, which could be responsible for a yet unknown mechanism [24,25].

Flavonoids show important anti-inflammatory, antioxidant, antiallergic, hepatoprotective, antithrombotic, antiviral, and anticarcinogenic activities, while also being typical phenolic compounds, thus acting as strong free radicals remover [26, 27]. Despite the described results became from an allopathic treatment, in the present *S. jambolanum* homeopathic study we observed significant metabolic improvement effects improving the homeostatic balance and metabolism [17].

Previous studies described that *S. jambolanum* lowers the risk of DM2 as improves insulin secretion, regulates glucose homeostasis-related enzymes [28, 29], maintains lipds levels and increases anti-oxidant enzymes activity [30, 31]. The likely anti-glication sample properties can be attributed to its known anti-oxidant potential [32-36]. Both total cholesterol and HDL changes observed in diabetic mice were reversed after homeopathic preparations [17]. Chemical compounds present in its formulation may increase HDL/LDL ratio through hepatocytes LDL-receptors activation, thus reducing plasma LDL levels [37, 38], results consistent with those found on the present work.

The reduction in *S. jambolanum* treated fasted mice blood glucose levels found in the present work are also consistent with previous studies [17, 28, 29 e 39], while another study with *Eugenia jambolanum* (EJ) aqueous extract potentiates rabbits pancreatic islets beta cells glucose-stimulated insulin secretion not unlike the tolbutamide effect. Moreover, flavonoids from the extract have been demonstrated to promote insulin secretion from insulinotrophic cells, thus reinforcing the hypothesis that this extract acts as a insulinotrophic agent itself [15]. This effect can be attributed to an increase in insulin production and/or secretion through pancreatic B cathapsin [40].

As for the insulin resistance, it is closely related to adipogenesis, or the increase in number and size of adipocytes, and this balance defines individuals' overall obesity level. Mature adipocytes release adipokines, such as TNFα, IL-6, leptin e adiponectin, e lipocin, so much so that, the greater the obesity and adisity, the higher the levels of such adipokynes, thus contributing to the establishment and worsening of insulin resistance [41] CCAAT/Enhancer Binding Protein (C/EBP-α) is a major central regulator of adipogenic processes [42] while Peroxisome Proliferator-Activated Receptor δ (PPARδ) act as a transcriptions activator of adipocytes-defining enzymes, mainly pertaining to insulin-dependent glucose transport, lipogenesis, lipolisis and adipokines synthesis [41,43].

In this sense, a lower C/EBPα expression would impair glucose tolerance by through lower GLUT4 expression, amongst others [44], which is in accordance with pour results, as we found an increase in C/EBPα in obese metformin group and HLHS SJ had better expression compared to the standard group, which may have been important in terms of glucose tolerance (Figure 5).

Finally, this *S. jambolanum* homeopathic formulation might lead to increased thermogenesis through increased UCP1 expression, as described in other studies [44,45,46] and as we found in the present work, wherein the obese mice treated with *S. jambolanum* presented and incrased expression of UCP1. What can be related to improve metabolism, thus protecting against obesity and other associated metabolic disorders. In addition, homeopathic effects are based on the body's energy, as already described [47]. Here we are showing for the first time that homeopathic treatment with *S. jambolanum* is able to modulate adipogenic and thermogenic related gene expression.

5. CONCLUSION

The present study indicates that oral administration of *S. jambolanum* homeophatic formulation to diet-induced obese mice ***S****yzygium* improves blood glucose, glucose tolerance, insulin sensitivity, adipocytes area and metabolic parameters in Diet-induced obese mice compared to the standard metformin treatment, possibly through induction o adipogenesis and thermogenesis related genes, thus directly affecting their weight loss and insulin sensitivity, thus suggesting *S. jambolanum* homeopathic formulation as safe and satisfactory complementary DM2 treatment.

Acknowledgments

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Author contributions

Authors give final approval of the version to be submitted.

Conflict of interest

The authors declare no conflict of interest.

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6. CONCLUSÕES

Em conclusão, os resultados obtidos mostram que a homeopatia de SJ e metformina reduziram a adiposidade em animais obesos induzidos por dieta e melhora de parâmetros metabólicos, glicemia, colesterol total e HDL em animais alimentados com dieta padrão, exercendo possíveis efeitos sobre marcadores moleculares da adipogênese,

Em conformidade com esses achados, a homeopatia demonstrou pontos positivos como medida complementar no controle da obesidade e tratamento do DM2, sob a perspectiva de uma maior divulgação acerca desses resultados e uma abordagem intervencional segura na clínica.

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ANEXO

ANEXO A – Parecer do Comitê de Ética e Pesquisa

Universidade Estadual de Montes Claros
Comissão de Ética em Experimentação e Bem-Estar Animal da Unimontes
CEEBEA

CERTIFICADO

Certificamos que o protocolo nº **176,** relativo ao projeto intitulado "*Efeito das preparações homeopáticas de Syzygium jambolanum e imposição de mão em camundongos no controle de diabetes mellitus tipo-2 induzido por dieta*" sob coordenação do Prof.º. Sérgio Henrique Sousa Santos, está de acordo com os princípios Éticos na Experimentação Animal, adotados pela Comissão de Ética em Experimentação e Bem-Estar Animal da Unimontes, e encontra-se APROVADO.A quantidade total de animais pelo CEEBEA para este projeto foi de 80 animais.
Este certificado é válido por cinco anos após sua aprovação.

Montes Claros, 05 de Dezembro de 2018.

Profª Drª Antonia de Maria Filha Ribeiro
Coordenadora do CEEBEA/UNIMONTES

ANEXO B – Normas para publicação no periódico Complementary therapies in clinical practice.

COMPLEMENTARY THERAPIES IN CLINICAL PRACTICE

GUIDE FOR AUTHORS

ISSN: 1744-3881

DESCRIPTION

Complementary Therapies in Clinical Practice is an internationally refereed journal published to meet the broad ranging needs of the healthcare profession in the effective and professional integration of complementary therapies within clinical practice.

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