RENNER PHILIPE RODRIGUES CARVALHO

EFEITOS DO TRATAMENTO COM EUGENOL EM MODELOS MURINOS HIPERGLICÊMICOS

Dissertação apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Biologia Celular e Estrutural, para obtenção do título de *Magister Scientiae*.

Orientadora: Mariana Machado Neves

Coorientadora: Graziela Domingues de Almeida Lima

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Assentimento:

Renner Philipe Rodrigues Carvalho
Autor

Mariana Machado Neves
Orientadora

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RESUMO

CARVALHO, Renner Philipe Rodrigues, M.Sc., Universidade Federal de Viçosa, junho de 2020. **Efeitos do tratamento com eugenol em modelos murinos hiperglicêmicos**. Orientadora: Mariana Machado Neves. Coorientadora: Graziela Domingues de Almeida Lima.

O diabetes é um dos maiores problemas de saúde enfrentados no mundo. Os tratamentos utilizados no controle da doença nem sempre são eficazes e podem apresentar efeitos colaterais. Nos últimos anos, estudos *in vitro* descreveram várias atividades positivas do eugenol no tratamento do diabetes. No entanto, ainda não está claro a característica bem como a magnitude dos seus efeitos em animais hiperglicêmicos. Portanto, neste estudo foi realizada uma revisão meta-analítica para determinar a magnitude do efeito do tratamento com eugenol sobre variáveis direta e indiretamente relacionadas ao diabetes. Esta revisão foi realizada a partir de uma busca sistematizada usando Web of Science, Scopus e PubMed como bancos de dados. Foram incluídos estudos publicados entre 1967 e 2019. Os termos 'diabetes' e 'glicose', 'hiperglicemia' e 'insulina' em combinação com 'eugenol', 'cravo' e seus nomes científicos foram pesquisados em títulos, resumos e palavras-chave. Posteriormente, os dados obtidos quanto a média, medidas de variância e N amostral foram avaliados quanto a modelos de efeitos aleatórios e mistos. Este estudo revelou efeitos fortes e negativos do eugenol sobre danos no fígado, perfil lipídico, níveis de glicose, dano oxidativo e dano renal. Por outro lado, o eugenol exerceu um efeito positivo no peso corporal e no sistema de defesa antioxidante. O efeito não é significativo na insulina e na atividade de enzimas do metabolismo dos carboidratos. Além disso, as análises de modelo misto revelaram que os efeitos do tratamento são significativamente influenciados pelo tipo de tratamento (eugenol puro ou extratos, óleos essenciais e cravo-da-índia em pó) e pela concentração administrada. Estes resultados mostram que o eugenol é um composto com potencial efeito terapêutico e pode ser um aliado no tratamento do diabetes, diminuindo os níveis glicêmicos, restaurando o status redox, regulando o perfil lipídico e atenuando os danos nos tecidos hepáticos e renais, e modulando o peso corporal.

Palavras-chave: Diabetes mellitus. Eugenol. Cravo. Animais experimentais.

ABSTRACT

CARVALHO, Renner Philipe Rodrigues, M.Sc., Universidade Federal de Viçosa, June, 2020. **Effect of eugenol treatment in hyperglycemic murine models.** Advisor: Mariana Machado Neves. Co-advisor: Graziela Domingues de Almeida Lima.

Diabetes is one of the biggest health problems faced worldwide. The treatments used to control the disease are not always effective and can have side effects. In recent years, *in vitro* studies have described various activities of eugenol in the treatment of diabetes. However, it is not yet clear whether the effects of this compound are negative, neutral, or positive for diabetic animals. Therefore, in this study, a meta-analytical review was carried out to determine the magnitude of the effect of eugenol treatment on variables directly and indirectly related to diabetes. This review included all studies published between 1967 and 2019. A systematic search was carried out using Web of Science, Scopus, and PubMed as databases. The terms 'diabetes' and 'glucose', 'hyperglycemia', and 'insulin' in combination with 'eugenol', 'cloves', and their scientific names were searched in titles, abstracts, and keywords. This study revealed strong and negative effects of eugenol on liver damage, lipid profile, glucose levels, oxidative damage, and kidney damage. Conversely, eugenol exerted a positive effect on body weight and the antioxidant defence system. The effect is not significant on insulin and enzyme activity in carbohydrate metabolism. In addition, the mixed model analyses revealed that the effects of treatment are significantly influenced by the type of treatment (pure eugenol or extracts and powdered cloves) and the concentration administered. These results show that eugenol is a compound with potential therapeutic effects and may be an ally in the treatment of diabetes, decreasing glycemic levels, restoring redox status, regulating the lipid profile, attenuating damage to liver and kidney tissues, and modulating the body weight.

Keywords: Diabetes mellitus. Eugenol. Clove. Experimental animals.

SUMÁRIO

INTRODUÇÃO GERAL

O diabetes mellitus é um dos maiores desafios da saúde pública moderna e, devido às altas taxas de prevalência, possui implicações sociais, financeiras e de desenvolvimento (ZIMMET *et al.*, 2014; OGURTSOVA *et al.*, 2017; CHO *et al.*, 2018). É um distúrbio metabólico multifacetado com a característica fundamental de hiperglicemia crônica. No diabetes tipo 1, a hiperglicemia ocorre devido à deficiência de insulina, como consequência da perda das células β das ilhotas pancreáticas (GEPTS, 1965; FLIER *et al.*, 1986; ATKINSON *et al.*, 2014; KATSAROU *et al.*, 2017). Com relação ao diabetes tipo 2, a hiperglicemia ocorre quando o *feedback* entre a ação e a secreção de insulina não funciona adequadamente. A ação da insulina em tecidos sensíveis é prejudicada pela resistência à sua ação, aumentando a produção de glicose no fígado e diminuindo a captação de glicose nos músculos e no tecido adiposo. Além disso, a secreção de insulina pelas células β das ilhotas pancreáticas também é prejudicada, sendo insuficiente para manter as concentrações normoglicêmicas (REAVEN, 1988; STUMVOLL *et al.*, 2005; ZHENG *et al.*, 2018).

O controle glicêmico é o principal objetivo no tratamento do diabetes (NATHAN, 2015). A terapia padrão para o tratamento do diabetes tipo 1 é o tratamento intensivo com insulina (ADA, 2015). Apesar disso, o grande grau de variabilidade da glicose e o risco de hipoglicemia da monoterapia com insulina têm estimulado a busca de terapias capazes de reduzir a necessidade da aplicação de insulina e os riscos associados (FRANDSEN *et al.*, 2016; WARSHAUER *et al.*, 2020). Terapias complementares ao tratamento com insulina poderiam ajudar a melhorar o controle glicêmico do paciente, além de apresentar efeitos independentes à redução da glicose, com consequente diminuição do risco de complicações do diabetes (LIVINGSTONE *et al.*, 2017).

Já as opções para o tratamento do diabetes mellitus tipo 2 incluem o tratamento inicial com monoterapia (geralmente com metformina), além de eventual adição de outros agentes orais. Em muitos pacientes, a terapia com insulina também é necessária para atingir níveis glicêmicos específicos (DEFRONZO, 1999; GARBER *et al.*, 2016; THRASHER, 2017). Assim como no tratamento do diabetes tipo 1, as terapias utilizadas no diabetes tipo 2 possuem efeitos colaterais, como hipoglicemia, perda de peso, insuficiência cardíaca, náuseas e diarreias (EL-KAISSI; SHERBEENI, 2011).

A limitação dos antidiabéticos atualmente disponíveis tem incentivado a busca por terapias alternativas para auxiliar o tratamento do diabetes de forma eficaz e mais segura (PANDEY *et al.*, 2011). Na medicina tradicional, mais de 1200 plantas são utilizadas por suas atividades hipoglicêmicas (MARLES; FARNSWORTH, 1995). A metformina, por exemplo, foi

desenvolvida a partir da espécie *Galega officinalis* e atualmente é um dos medicamentos mais utilizados no tratamento do diabetes (PANDEY *et al.*, 2011).

O cravo-da-índia (*Syzygium aromaticum*) tem mostrado bons resultados em estudos *in vitro* (PRASAD *et al.*, 2005; ADEFEGHA; OBOH, 2012), *in vivo* (SHUKRI *et al.*, 2010; ADEFEGHA *et al.*, 2014) e em pacientes diabéticos tipo 2 (KHAN *et al.*, 2006). As propriedades antidiabéticas do cravo têm sido atribuídas principalmente ao eugenol (SINGLETARY, 2014). De fato, *in vitro*, o eugenol foi capaz de inibir as enzimas α-amilase pancreática e lipase (MNAFGUI *et al.*, 2013), inibir a formação de produtos finais de glicação avançada (AGEs) (SINGH *et al.*, 2016), além de inibir a produção de glicose hepática (JEONG *et al.*, 2014). Essas atividades resultaram em redução das concentrações glicêmicas em estudos com animais experimentalmente induzidos ao diabetes (SINGH *et al.*, 2016; AL-TRAD *et al.*, 2019). O eugenol é geralmente reconhecido como seguro pela Organização Mundial da Saúde (OMS) e amplamente utilizado na medicina tradicional (KHALIL *et al.*, 2017). Na indústria de alimentos, é usado como conservante e como agente aromatizante para alimentos e cosméticos (BARBOZA *et al.*, 2018). Apesar de seguro e de seu uso amplamente difundindo, não existem estudos clínicos avaliando os efeitos do eugenol no tratamento da doença, seja como terapia única ou complementar.

Até o presente momento, não foram encontrados estudos avaliando o efeito do eugenol sobre parâmetros testiculares e epididimários de animais diabéticos. Este era o principal objetivo do projeto de mestrado, considerando que o grupo vem trabalhando com modelos diabéticos (SOUZA *et al.*, 2018; SERTORIO *et al.*, 2019), avaliando o efeito nos órgãos reprodutores a partir da exposição a contaminantes ambientais (SOUZA *et al.*, 2019), bem como o uso de extratos vegetais como tratamento do diabetes (ERVILHA *et al.,* 2020 em fase de correção). Sabe-se que a diabetes causa problemas reprodutivos importantes, devido a redução das concentrações séricas de testosterona, com consequentes danos à espermatogênese, qualidade espermática e ereção (BALLESTER *et al.*, 2004; AMARAL *et al.*, 2006; SCARANO *et al.*, 2006; BAL *et al.*, 2011; GOBBO *et al.*, 2015). Portanto, o objetivo geral deste trabalho foi de caracterizar, como base na literatura, a ação do eugenol como agente terapêutico do diabetes, bem como de problemas relacionados com a fertilidade masculina decorrente dessa doença. Neste contexto, realizou-se um estudo meta-analítico sobre o efeito do eugenol no tratamento da diabetes em modelos animais. Posteriormente, seria realizado um experimento *in vivo* com o objetivo de avaliar os efeitos do tratamento com eugenol sobre os parâmetros testiculares, epididimários e espermáticos de ratos Wistar induzidos experimentalmente ao diabetes. Este estudo confirmaria ou não nossa hipótese de que o eugenol afeta positivamente parâmetros

reprodutivos em animais diabéticos. O trabalho foi iniciado em janeiro de 2020, mas, devido ao estado de pandemia causado pela COVID19 e ao período de isolamento social, a parte experimental *in vivo* tornou-se inviável.

Neste contexto, o presente trabalho apresentará uma revisão meta-analítica sobre o efeito do eugenol em animais hiperglicêmicos, com os seguintes objetivos:

- Determinar a magnitude do efeito do tratamento com eugenol sobre diversas variáveis relacionadas a glicemia, biometria corporal, parâmetros bioquímicos, danos teciduais e marcadores do estresse oxidativo em animais induzidos experimentalmente ao diabetes;
- Comparar a magnitude dos efeitos do eugenol sobre essas variáveis considerando as diferenças metodológicas entre os estudos. Os resultados desse estudo visam ajudar a entender o potencial do eugenol como um antidiabético a ser utilizando como tratamento complementar, bem como suas implicações para a saúde humana.

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ARTIGO

Effect of eugenol treatment in hyperglycemic murine models: A meta-analysis

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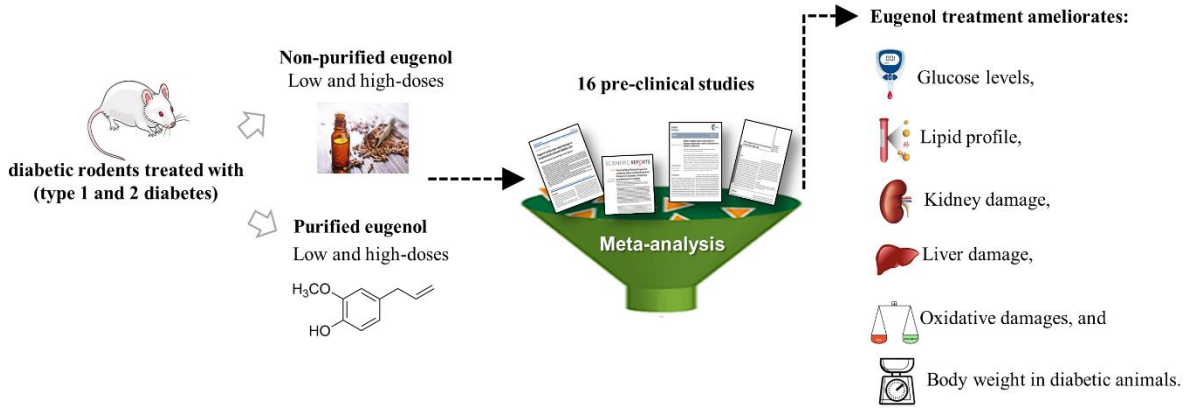
Graphical Abstract



Abstract

Diabetes is a highly prevalent health condition affecting many people worldwide. In vitro studies have described the positive effects of cloves and its major compound, eugenol, in the treatment of diabetes. However, it is unclear whether the effects of this compound are negative, neutral, or positive, on hyperglycemic animals. Therefore, a meta-analytical review was conducted to determine the magnitude of effects of eugenol on variables directly and indirectly related to diabetes. This study revealed that eugenol treatment decreased the glucose levels and the activity of carbohydrate-metabolizing enzymes, ameliorated the lipid profile, and reduced the oxidative, renal, and hepatic damages in hyperglycemic rodents. Moreover, eugenol alleviated the weight loss and restored the activity of the antioxidant defense system. Insulin levels was not affected by eugenol treatment. Also, mixed model analyses revealed that the use of purified or non-purified eugenol and the concentrations administered significantly affected the treatment outcome. In conclusion, our findings indicate that eugenol may have potential therapeutic effects in the treatment of diabetes. Furthermore, this study can direct future preclinical and clinical trials, with important implications for human health.

Keywords: Syzygium aromaticum; clove; diabetes mellitus; *in vivo* studies; glucose; rodents.

Chemical compound studied in this article: Eugenol (PubChem CID: 3314).

1. Introduction

Diabetes mellitus is one of the most significant health challenges of the 21st century [1]. Overall, type 1 and 2 diabetes result in high blood glucose concentrations which, in turn, cause structural and functional changes in organs and alter the metabolism of carbohydrates, lipids, and proteins [2,3]. Diabetes mellitus is often associated with liver dysfunction, diabetic nephropathy, and dyslipidemia [4–6]. Hyperglycemic conditions increase the generation of reactive oxygen species (ROS), which can also lead to tissue damage induced by oxidative imbalance [7–9].

To control glycemia is the main objective of a diabetes treatment. Although the drugs used to treat diabetes are exceptionally effective, they cannot be administered to all patients. Insulin treatment can cause a high degree of glucose variability, increasing the risk of hypoglycemia [10]. Likewise, hypoglycemia, weight loss, heart failure, and intestinal disorders are common side effects associated with continued use of medications such as biguanides, meglitinides, and sulfonylureas [11]. Moreover, in some patients, monotherapy is not effective in glycemic control and therefore, combined use of drugs becomes necessary [12]. With regard to this, development of more effective and affordable therapies for the prevention and treatment of diabetes is crucial [11,13,14].

In the context of diabetes treatments, several phenolic compounds are known to contribute to the management of hyperglycemia and balance of redox status in individuals with diabetes. Recently, a review of clinical studies showed that consumption of (poly) phenols reduced glucose levels of individuals with type 2 diabetes [15]. Research on pharmacological properties of eugenol, a polyphenol, has been the focus of recent studies [16–19]. Eugenol is found mainly in essential oils and clove extracts (*Eugenia caryophyllata*, also known as *Syzygium aromaticum*).

Several *in vitro studies* have demonstrated that eugenol inhibits the activity of enzymes associated with energy metabolism in assays using clove extracts [20–23] and purified eugenol [24]. Eugenol possibly increases the uptake of 2-deoxyglucose via the phosphoinositide 3-kinase

(PI3K)-dependent pathway [25]. Other *in vitro* studies have shown that clove extract may act as an antioxidant by inhibiting of protein glycation and lipid peroxidation [26,27].

Nevertheless, there is a paucity of information regarding the therapeutic efficacy of eugenol in clinical trials. A clinical study with type 2 patients with diabetes concluded that clove could be used as an anti-hyperglycemic food supplement [28]. In animal studies, clove intake showed anti-hyperglycemic, hypolipidemic, and antioxidant properties under hyperglycemic conditions [29,30]. In addition, treatment of diabetic animals with pure eugenol also reduced blood glucose levels, restored antioxidant potential, and attenuated oxidative damage [31,32].

Currently, to the best of our knowledge, there is no systematic review of the activity of eugenol in experimental models of diabetes in literature. Thus, this study was designed to meta-analytically review the *in vivo* preclinical effects of eugenol (including clove) in a diabetic animal model to assess these aspects and contribute to an improvement in diabetes treatment.

2. Methods

2.1. Focus question

The main question we sought to answer in this systematic review is what the magnitude of the effects of eugenol treatment is in a diabetic murine model.

2.2. Protocol and registration

The present review was conducted according to the Preferred Reporting Items for Systematic Review and Meta Analyses (PRISMA) statement [33]. This meta-analysis was registered at PROSPERO, International Prospective Register of Systematic Review with registration number CRD 42019157503 (http://www.crd.york.ac.uk/PROSPERO).

2.3. Search strategy

This meta-analysis involved an extensive review of the effects of eugenol treatment on

diabetic animal models. All original full-text studies published up to 2020 were included in the review. A systematic search of the Web of Science, Scopus, and PubMed/Medline databases was conducted. The search was performed electronically using the following terms in titles, abstracts, and keywords: “diabetes,” ‘glucose,” “hyperglycemia,” and “insulin” in combination with “eugenol,” “clove,” “*Eugenia caryophyllata*,” “*Syzygium aromaticum*,” and “*Eugenia aromatica*.” To expand the search, the wildcard symbol “*” was used.

2.4. Studies reviewed

The following criteria were previously established for including studies in such reviews: 1) published in peer-reviewed journals, 2) published in the English language, 3) rats and mice were used as animal models of induced diabetes , 4) experimental studies must present control (diabetic animals) and treatment (diabetic animals treated with eugenol), and 5) studies that reported means, sample size, and a measure of variance (standard deviation, standard error or confidence intervals) for both control and treatment groups.

Studies with unclear sample sizes for experimental groups were excluded from this meta-analysis. Other studies were excluded if, 1) the journal impact factor was ≤ 1.0, 2) the study used species other than rats and mice as animal models, 3) animals were treated with compounds or extracts other than clove or eugenol, and 4) were secondary studies (reviews, conference proceedings, and commentaries). The search was complemented using the reference lists of the included studies.

Response mean values ($X^{control}$ and $X^{treatment}$), standard deviations ($S^{control}$ and $S^{treatment}$), and sample size ($N^{control}$ and $N^{treatment}$) were retrieved from the text, tables, or figures, or both, from each study included in this review. Data available in figures were digitized and the means and variances were obtained using ImageJ software [34] after calibrating each picture to the nearest 0.01 mm. Measurements of variance were all converted to standard deviations of the mean using MetaWin statistical calculator [35].

We performed a separate meta-analysis of each of the following nine variables affected by eugenol in animals with diabetes: 1) glucose levels, 2) insulin levels, 3) carbohydrate-metabolizing enzymes, 4) body weight, 5) antioxidant defense, 6) oxidative damage, 7) lipid profile, 8) liver damage, and 9) kidney damage. The parameters included for each variable are shown in the Table 1. Only variables that generated at least five independent comparisons were included in the analysis. Moreover, according to the information provided by the authors, the mixed-model effect of the meta-analysis was used to categorize the studies based on the following methodological moderators: i) eugenol source (purified or non-purified eugenol); ii) concentration administered (low- or high-dose: 0.01 to 10 mg kg^{-1} and < 10 mg kg^{-1}, respectively), and iii) type of diabetes (type 1 or type 2 diabetes).

2.5. Meta-analysis

The standardized difference between control and treatment groups was used to interpret and summarize the effects of eugenol treatment in diabetic animals. For each study, the magnitude of effect (d) was calculated as follows: $d = (X_t - X_d/SD) \times J$, where X_t and, X_d are the responses of the treatment and diabetic control group, respectively, SD is the pooled standard deviation, and J is a correction term to remove small sample size bias [35]. After Hedge's d was calculated for each independent comparison, the cumulative effect (d++) of each variable surveyed was calculated using a random-effect model.

We used the mixed-effect model to analyze the methodological moderators where it assumes that differences between studies within a class are determined by sampling errors and random variation. Upper and lower confidence intervals (CI) were calculated according to the average cumulative effect, and intervals that did not overlap with zero, with n-1 degrees of freedom (df), were considered significant. Conventionally, d++ values around 0.2, 0.5, and 0.8 are considered to indicate weak, moderate, and strong effects, respectively [36]. Moreover, a

positive and negative *d* ++ value indicate that the treatment effect increased and decreased the measured variable values, respectively.

Table 1.

Parameters collected from the eligible studies and grouped into nine variables analyzed under random-effect model for meta-analysis review.

Variables	**Parameters**
Glucose levels	Blood glucose levels.
Insulin levels	Insulin levels.
Carbohydrate-metabolizing enzymes	Activity of alpha-amylase and alpha-glucosidase.
Body weight	Body weight.
Antioxidant defense	Activity of ascorbic acid, glutathione peroxidase, reduced glutathione, catalase, Superoxide dismutase, glutathione–s–transferase, glutathione reductase, thioredoxin reductase.
Oxidative damage	Levels of hydroperoxides, nitric oxide, protein carbonyls, total thiols, dichlorofluorescein, malondialdehyde.
Lipid profile	Triglycerides, cholesterol, and low-density lipoprotein-cholesterol.
Liver damage	Concentrations of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total-bilirubin, and gamma-glutamyl transferase.
Kidney damage	Values of blood urea nitrogen, the activity of creatine kinase, activities and creatinine, urea, uric acid, glomerular damage, mesangial matrix index, glomerular filtration rate, and urine protein.

Heterogeneity analyses (Q statistic) were used to determine whether categorical groups in mixed models were homogeneous with respect to calculated effect sizes. We calculated the

total heterogeneity (Q_t) of all effects tested and the Q within (Q_w) and between (Q_B) groups. The significance of these statistics was evaluated using a chi-square distribution with n-1 df. Our analyses were based only on published studies; studies that show large and significant effects may be more likely to get published than those showing weak or no effects, and this phenomenon is referred to as the "file-drawer problem," *sensu* [37]. Consequently, we calculated fail-safe numbers for each effect tested. Fail-safe numbers indicate the number of non-significant, unpublished, or missing studies that would need to be added to the sample to change the results from significant to non-significant [35]. As a rule of thumb, fail-safe results are considered robust if the fail-safe number exceeds $5k + 10$ [38], where k is the number of comparisons in the analysis. All analyses were conducted using MetaWin 2.1 software [35], whereas the graphics were created using Sigma Plot 10.0 software [39].

3. Results

The flow diagram summarizing the literature search process is shown in Figure 1. The initial search resulted in a collection of 774 articles from PubMed/MEDLINE, Web of science, and Scopus databases. After removing articles that were in duplicates or met the exclusion criteria, 16 studies were considered for meta-analysis (Fig. 1).

3.1. Qualitative data

The publication characteristics of eligible studies are summarized in Table 2. From the included 16 studies, 68.8% used laboratory animal models of induced type 1 diabetes, whereas 31.2% used type 2 diabetes-induced models. Diabetes was mainly induced using streptozotocin (STZ, 56.3%), followed by alloxan (12.5%), or high-fat diet (HFD, 6.2%). For STZ, only one study administered consecutive doses (45 mg kg-1 for 5 days), in contrast to the other studies which administered single doses at concentrations between 30 and 55 mg kg^{-1}. Additionally, 12.5% of the studies used STZ in combination with HFD and 12.5% used genetically modified animals.

HFD, genetically modified diabetic animals, and STZ-HFD protocols were used to induce type 2 diabetes. Further, rat models (68.8%) were mainly used in these studies, followed by mouse models (31.2%). Purified eugenol was tested in 50% of the studies, whereas the other 50% investigated non-purified eugenol, of which 31.25% used extracts, 12.5% used clove powder, and 6.25% used clove oil.

The concentration of eugenol provided varied between 2.5 and 657 mg kg^{-1} as based on the eugenol source, route of administration, and dosing frequency (Table 2). Eugenol was mainly administered orally (68.8%), followed by intragastrically (18.7%), and intraperitoneally (12.5%). The dosing frequency was daily (87.5%), twice a week (6.25%), and on alternate days (6.25%).

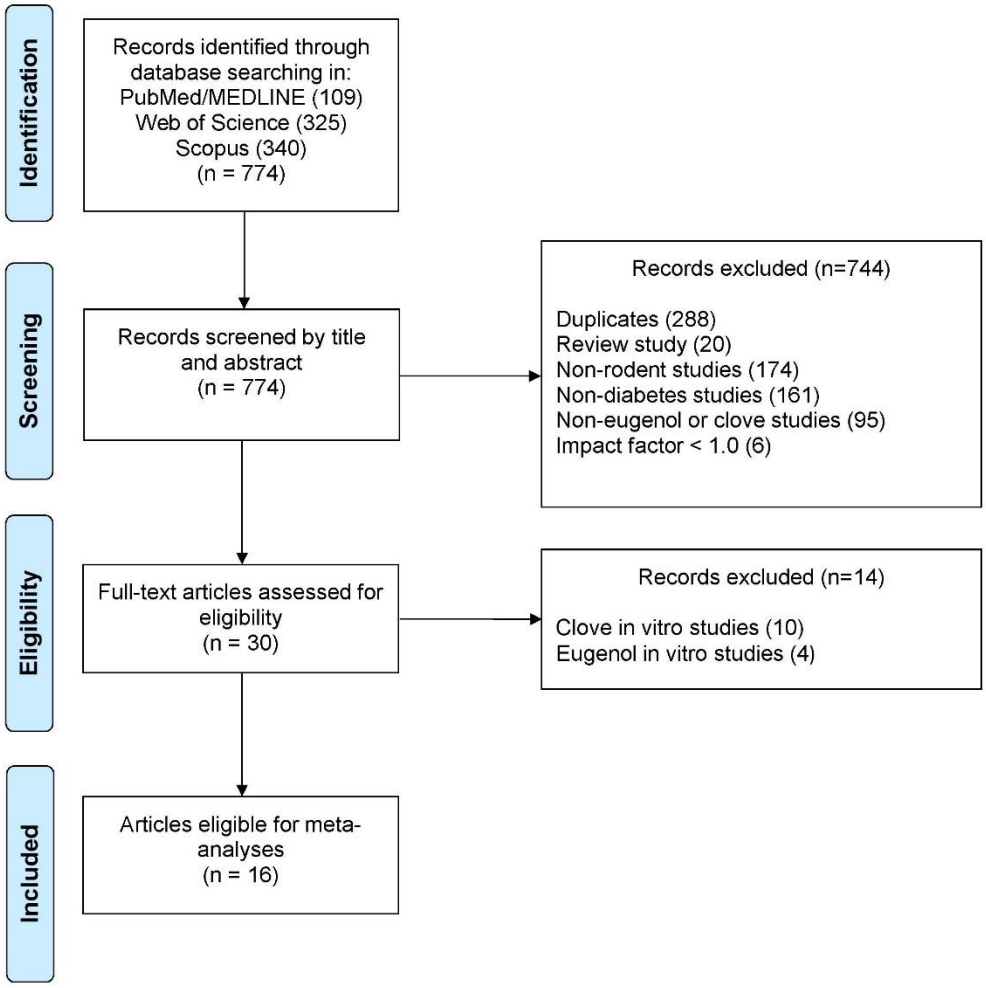


Fig. 1. Flow diagram of search results to define the articles to be included in meta-analysis review, according to PRISMA (Preferred Reporting Items for Systematic Review and Meta-Analysis.

Table 2.

Characterization of studies used in the meta-analysis. Information on type of diabetes (TD), species and lineage, number of animals per group (N), treatment description, and treatment time.

Authors (Year)	Diabetes type	Species (lineage)	N	Treatment type	Treatment description	Treatment time (days)
Adefegha *et al.* (2014)	T2D (35 mg STZ* + HFD)	Rat (Wistar)	6	Clove powder	1.7 and 3.7 g kg^{-1} bw; daily; oral route.	4, 7, 10, 13, and 30
Akila *et al.* (2018)	T1D (55 mg STZ*)	Rat (Wistar)	8	*Syzygium aromaticum* aqueous extract	2 g kg^{-1} bw; daily; gavage route.	7, 14, 21, and 28
Al-Trad *et al.* (2019)	T2D (40 mg STZ* + HFD)	Rat (Sprague-Dawley)	10	Purified eugenol (Sigma Aldrich)	10 mg kg^{-1} bw; daily; oral route.	45
Chowdhury *et al.* (2016)	T1D (120 mg alloxan*)	Mice (Swiss)	4	*Syzygium aromaticum* methanolic extract	200 and 400 mg kg^{-1} bw; daily; oral route.	1, 2, 3, and 4
Garud and Kulkarni (2016)	T1D (55 mg STZ*)	Rat (Sprague Dawley)	8	Purified eugenol (Sigma Aldrich)	5 and 10 mg kg^{-1} bw; daily; oral route.	28 d

Jeong *et al.* (2014)	T2D (HFD)	Mice (C57BL/6J)	8	Purified eugenol (Sigma Aldrich)	20 and 40 mg kg^{-1} bw; daily; oral route.	105 d
Kiani *et al.* (2018)	T1D (55 mg STZ*)	Rat (Wistar)	8	*Syzygium aromaticum* ethanolic extract	75 mg kg^{-1} bw; daily; oral route.	28 d
Kuroda *et al.* (2012)	T2D (genetic mice)	Mice (KK-Ay)	5	*Syzygium aromaticum* ethanolic extract	657 mg kg^{-1} bw; daily; oral route.	21 d
Mnafgui *et al.* (2013)	T1D (150 mg alloxan*)	Rat (Wistar)	8	Purified eugenol (Sigma Aldrich)	80 mg kg^{-1} bw; daily; gastric gavage route.	30 d
Nangle *et al.* (2006)	T1D (42.5 mg STZ*)	Rat (Sprague Dawley)	22 CG/ 44 TG	Purified eugenol (Sigma Aldrich)	200 mg kg^{-1} bw; daily; oral route.	14 d
Prasad *et al.* (2016)	T1D (55 mg STZ*)	Rat (Wistar)	6	Purified eugenol (Sigma Aldrich)	10 mg kg^{-1} bw; alternate days; intraperitoneal route.	42 d
Sanae *et al.* (2014)	T2D (genetic mice)	Mice (C57BLKS/J db+/db+)	6	*Syzygium aromaticum* ethanolic extract	Diets supplemented, with 5% or 10%; daily; oral route.	28 d

Shukri *et al.* (2010)	T1D (50 mg STZ*)	Rat (Sprague Dawley)	8	Clove powder	Powdered cloves (equivalent to 100 mg kg^{-1} bw of eugenol); daily; oral route.	21, 42, 63, 84, and 105 d
Singh *et al.* (2016)	T1D (45 mg STZ/ 5d)	Mice (BalbC)	8	Eugenol isolated from *Ocimum gratissimum*	100 mg kg^{-1} bw; twice a week; intraperitoneal route.	14 d
Srinivasan *et al.* (2013)	T1D (40 mg STZ*)	Rat (Wistar)	6	Purified eugenol (Sigma Aldrich)	2.5, 5, and 10 mg Kg^{-1} bw; daily; intragastric route.	30 d
Zari and Al-Attar (2007)	T1D (30 mg STZ*)	Rat (Wistar)	10	Clove oil	5% clove oil in the diet; daily; oral route.	7 and 15 d

STZ: Streptozotocin; HFD: High fat diet; bw: body weight; d: day(s); *Single dose. CG: control group; TG: treatment group.

3.2. Meta-analysis

Eugenol treatment exhibited strong effects by ameliorating the liver damage, glucose levels, lipid profile, oxidative damages, and kidney damage. It also inhibits the activity of carbohydrates-metabolizing enzymes. Moreover, eugenol exerted a strong and positive effect on antioxidant enzymes activity. Body weight was moderately ameliorated by treatment (Fig. 2). Rosenthal's fail-safe numbers for these effects were fairly high, relative to the number of independent comparisons (Supplementary Table 1). Finally, eugenol did not significantly affect the insulin levels (Fig. 2).

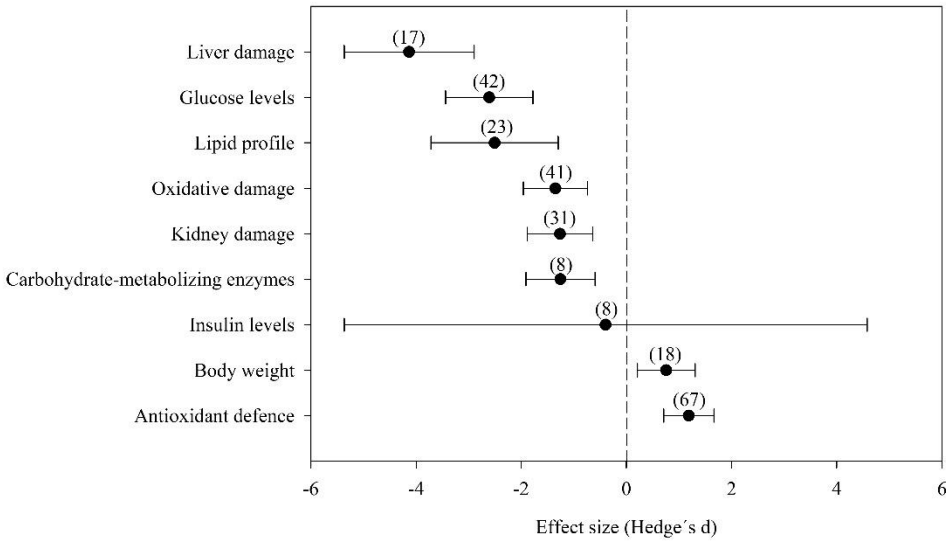


Fig. 2. Effects of eugenol treatment on variables in diabetic animals. The cumulative effect size is reported with its 95% confidence interval and effects are significant if confidence intervals do not overlap with zero. (n) = number of independent comparisons.

Eugenol source was the main methodological moderator influencing the variables analyzed in this study, followed by eugenol concentrations and type of diabetes. Purified eugenol showed a stronger effect in reducing liver and kidney damage than that of non-purified eugenol (Fig. 3). Purified eugenol also exhibited a strong effect in improving the antioxidant defense, body weight, and lipid profile (Fig. 3). Moreover, eugenol concentrations ≤ 10 mg kg^{-1} elicited the strongest effects, restoring the antioxidant defense system, body weight (Fig. 3), and increasing insulin levels (d_{++} = -249.45, df 3, CI = -379.44 to -119.46). Finally, treatment in type 2 diabetic animals had the strongest effect in decreasing blood glucose levels, while liver damage was most decreased in type 1 diabetic animals (Fig. 3). The results of methodological moderators that did not show significant effect or were not analyzed under the mixed effect model are shown in the Supplementary Table 2.

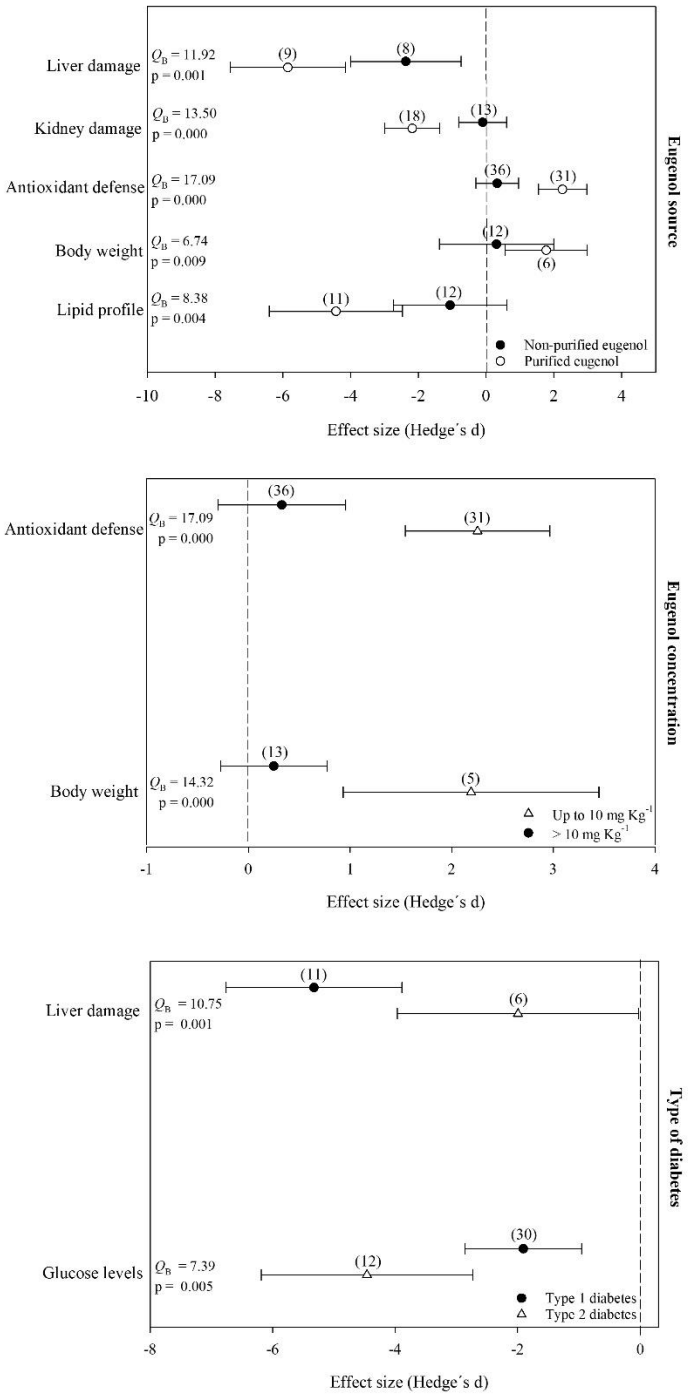


Fig. 3. Effects of the eugenol source (purified eugenol and non-purified eugenol), eugenol concentrations (up to 10 or > 10 mg Kg^{-1}), and type of diabetes (type 1 and type 2) on liver damage, kidney damage, antioxidant defense, body weight, lipid profile and glucose levels in diabetic animals. The cumulative effect size is reported for each effect measured with its 95% confidence intervals, and effects are significant if confidence intervals do not overlap with zero. Numbers in parentheses indicate the number of independent comparisons for each effect. Q_B indicates heterogeneity between groups.

4. Discussion

The findings of the current review revealed that eugenol strongly attenuated functional and structural damages caused by hyperglycemia in animal models. Herein, we observed that eugenol ameliorated the glucose levels, lipid profile, body weight, and reestablished the antioxidant defense. Also, eugenol decreased the markers of oxidative, hepatic, and renal damage in rodent models with experimentally induced diabetes. These results reinforced the idea that this polyphenol compound has promising therapeutic effects on hyperglycemia. Moreover, we propose eugenol as a potential candidate for clinical trials targeted at improving diabetes treatment. Undoubtedly, diabetes is a severe disease that affects millions of people worldwide [12,40,41], and any effort to enhance its treatment is relevant. Diabetes is a chronic metabolic disorder related to a deficiency in insulin secretion, the inability of body systems to use this hormone efficiently, or both [42], which is associated with a wide range of additional complications, such as renal diseases, liver diseases, infections, and cancers [43].

In this study, we showed that eugenol reduced glucose levels of diabetic animals. The effect of eugenol on the activity of carbohydrate-metabolizing enzymes, such as alpha-amylase and alpha-glucosidase, may have contributed to this finding (Fig. 4a). Eugenol can inhibit glucose uptake by intestinal cells, which decreases postprandial hyperglycemia [44] and prevents the onset of late diabetic complications [45]. This inhibition occurs due to the ability of its hydroxyl groups to interact with active sites of enzymes and delay the carbohydrate absorption [21,46–50]. Notably, in the small intestine, pancreatic alpha-amylase is related to the breakdown of long-chain carbohydrates, whereas intestinal alpha-glucosidase ends this digestive process by transforming these oligosaccharides into monosaccharide glucose [51,52]. Accordingly, enterocytes can uptake glucose by the sodium-glucose cotransporter 1, which is constitutively localized to the apical brush border membrane [53]. Therefore, inhibition of carbohydrate-metabolizing enzymes may be the first effect of eugenol on glucose metabolism in treated diabetic animals.

In addition to the above, eugenol may reduce the glucose levels in hyperglycemic rodents by acting on the phosphorylation of Ca^{+2}-calmodulin-dependent protein kinase kinase (CAMKK) and AMP-activated protein kinase (AMPK). On the one hand, the activation of the CAMKK-AMPK pathway can inhibit glucose production in the liver, suppressing the CREB-regulated transcription coactivator 2 – cAMP-response element-binding protein complex (CRTC2-CREB) activation, and decreasing the expression of gluconeogenic enzymes [54,55] (Fig. 4b). Hepatic glucose production is increased under diabetic conditions, leading to progressive hyperglycemia [54,56]. Thus, the inhibition of this glucose pathway is an efficient mode of action of eugenol to ameliorate the glycemia in diabetic animals. On the other hand, this compound may stimulate glucose uptake by an insulin-independent pathway. Eugenol stimulates glucose transporter-4 (GLUT4) translocation from the intracellular storage depots to the plasma membrane of skeletal muscle cells by AMPK phosphorylation, promoting glucose transport [32,57] (Fig. 4b). GLUT4 is the main protein involved in glucose uptake influenced by insulin. In the case of insulin resistance, glucose uptake may be disturbed in muscle cells increasing serum glucose levels [57]. It is noteworthy that the glycemia reported in eugenol-treated diabetic animals reached similar levels than those observed in their controls, as well as the levels exhibited by diabetic animals treated with metformin that has been the mainstay of therapy for diabetes mellitus [32]. Fortunately, this glucose reduction by eugenol did not cause hypoglycemia in treated-diabetic animals [31,58], which could be a side effect. Altogether, our findings indicate that eugenol plays an anti-hyperglycemic role by decreasing serum glucose levels probably involving three modes of action.

This meta-analytical review showed that eugenol enhanced the lipid profile of diabetic animals. Serum levels of cholesterol, triglyceride, and low-density lipoprotein (LDL) are known to increase in diabetic animals [29,32,59]. Studies have shown that phenolic groups, such as those in the chemical structure of eugenol, modulate the activity of different kinases, such as protein kinase A (PKA) [60]. Under hyperglycemic or insulin-deficient conditions, PKA activates the

hormone-sensitive intracellular lipase and promotes the release of non-esterified fatty acids from triglycerides stored in adipose tissue. As a result, there is an increase in the production of hepatic triglycerides [61–63]. Eugenol, in turn, may bind to proline-rich regions of the β subunits of PKA and inhibit its activity by decreasing that of hormone-sensitive intracellular lipase [64]. Consequently, low levels of non-esterified fatty acids are available for triglycerides production by the liver, influencing the serum lipid profile (Fig. 4c).

Moreover, eugenol restored the activity of antioxidants and mitigated oxidative damages, which are both mediated by the cellular redox process. Studies included in this review reported an increase in the activity of enzymatic (e.g., superoxide dismutase [SOD], catalase [CAT], thioredoxin reductase, and reduced glutathione [GSH]-related enzymes) and non-enzymatic antioxidants (e.g. ascorbic acid) analyzed in blood samples and pancreatic, brain, cardiac, renal, and hepatic tissues of diabetic animals after eugenol treatment. By contrast, the latter decreases the generation of oxidative byproducts, such as hydroperoxides, protein carbonyls and malondialdehyde [29,32,65,66]. Typically, hyperglycemia elevates ROS production and inhibits the activity of antioxidants through non-enzymatic glycation of some protein regions of enzymes such as CAT and SOD, contributing to the progression and development of diabetes and its complications [7,67–72]. In the polyol pathway, glucose is reduced to sorbitol by the enzyme aldose reductase in a reduced nicotinamide adenine dinucleotide phosphate (NADPH)-dependent manner. Thus, high glycemic levels decrease the availability of NADPH, which is essential for GSH production [72]. Once eugenol acts as an anti-hyperglycemic agent, low levels of glucose might inhibit the occurrence of non-enzymatic glycation and restore SOD and CAT activity. This anti-hyperglycemic action also affects negatively the glucose reduction within the polyol pathway, increasing the levels of NADPH used for GSH production (Fig. 4d). Aside from the anti-hyperglycemic activity, eugenol acts as a potent antioxidant [73]. Its phenolic hydroxyl groups bind to free radicals generated during the oxidative stress by donating a hydrogen

molecule (Fig. 4e). Hence, it promotes the stability of these radicals, minimizing their damage [74–76].

Our findings demonstrated that eugenol attenuated the deleterious effects of hyperglycemia on liver tissue (Fig. 4). This effect was determined by measuring biochemical parameters such as alanine transaminase, aspartate transaminase, and alkaline phosphatase, which are commonly assessed to evaluate hepatic function. In hyperglycemic animals, serum levels of aminotransferases may increase due to cellular damage, because subtle changes in the membrane may cause the intracellular enzymes to pass to the extracellular space [46,77,78]. In addition, elevations in the serum levels of bilirubin and gamma-glutamyl transferase reflect hepatocyte damage caused by diabetes [79,80]. Studies have reported that eugenol protected hepatic tissue against injury induced by thioacetamide [18], hepatic steatosis [81], and hypercholesterolemia [82]. Additionally, among antioxidant compounds, phenolic compounds protect the liver and reduce liver enzyme levels in diabetes [83]. Therefore, the antioxidant activity of eugenol as reported here, appears to be the mechanism mediating its protection of the liver tissue. This effect was also observed as a reduction of histopathological damage of the liver of diabetic animals treated with eugenol [59].

With regard to kidney damage, diabetes commonly causes disturbance in homeostasis of glucose and insulin, which may be aggravated by oxidative stress and other disease-related factors [65,84,85]. The amelioration of kidney function related to renal clearance, urea levels, and creatinine parameters may be a consequence of the reestablishment of glucose levels and redox status by eugenol [65,86–89] (Fig. 4). Therefore, the therapeutic effect of eugenol has once again been confirmed by the reduction in these parameters. In addition, the reduction in histopathological kidney damage also suggests an improvement in renal function [65].

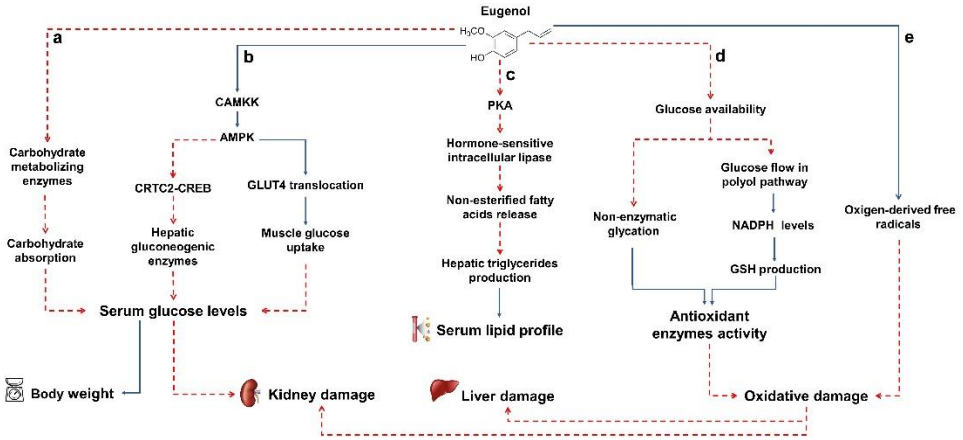


Fig. 4. Schematic representation of potential biological pathways of eugenol in the treatment of diabetes. Eugenol reduces serum glucose levels in diabetic animals by a) inhibition of carbohydrate-metabolizing enzymes that, in turn, reduces carbohydrate absorption by intestinal cells; b) activation of the CAMKK-AMPK pathway that inhibits the glucose production in the liver via suppression of the CRTC2-CREB complex. The CAMKK-AMPK pathway can also stimulate GLUT4 translocation, which results in higher glucose uptake by skeletal muscle cells. In the end, low levels of glucose ameliorate the body weight loss and kidney damages of diabetic animals. Moreover, eugenol ameliorates c) the serum lipid profile of diabetic animals by inhibiting PKA activity and decreasing hormone-sensitive intracellular lipase activity. The latter decreases the non-esterified fatty acids release by adipose tissue, which reduces the production of hepatic triglycerides. d) The reduced glycemia prevents the glycation of antioxidant enzymes, as well as reduces the glucose flow in the polyol pathway, with a consequent increase of NADPH levels required for GSH production. These both pathways enhance the activity of antioxidant enzymes. In addition, e) phenolic hydroxyl groups from eugenol molecule can bind to oxygen-derived free radicals by donation of a hydrogen molecule, which promotes the stability of these free radicals. Altogether, the mode of action of eugenol on the antioxidant defense system reduces oxidative damages caused by oxidative stress, alleviating the damages on liver and kidney tissues. Dashed red lines: inhibition via; Solid blue lines: activation; CAMKK: Ca^{+2}-calmodulin-dependent protein kinase kinase; AMPK: AMP-activated protein kinase; CRTC2-CREB: CREB-regulated transcription coactivator 2- cAMP-response element-binding protein complex; GLUT4: glucose transporter-4; PKA: protein kinase A; NADPH: nicotinamide adenine dinucleotide phosphate; GSH: reduced glutathione.

Eugenol treatment ameliorated the body weight probably due to its activity in the glycemic control (Fig. 4). Weight loss in experimental diabetes can be due to the unavailability of carbohydrates for energy metabolism or the loss and degradation of selected proteins [90]. Additionally, excessive protein catabolism to provide amino acids for gluconeogenesis during insulin reduction is known to result in muscle mass and weight loss in non-obese diabetic rats [58]. In these processes, eugenol appears to promote the restoration of efficient metabolic homeostasis and improve the health of animals.

The non-significant effect of eugenol on insulin levels indicated that it may not directly affect insulin. Although the random model did not show significant differences with insulin concentrations. Although STZ and alloxan are highly toxic to β cells, the effect is short-lived and when there are surviving cells, chronic hyperglycemia can be a toxic factor [91]. The increase in insulin levels in diabetic animals treated with eugenol may be due to the anti-hyperglycemic potential of eugenol, mediated by its enhancement of increased secretion of existing residual β-cells [32,59,92]. However, we believe that this effect does not occur in human diabetes because while experimental diabetes provides models for studying the disease, it is not equivalent to the human disease condition.

4.1. The influence of methodological moderators on eugenol effects

According to our mixed-effect model, the variables were mostly influenced by eugenol source, followed by eugenol concentrations and type of diabetes. In the first methodological moderator, the purified eugenol caused beneficial effects in diabetic animals on their body weight, antioxidant defense, lipid profile, liver damage, and kidney damage. Indeed, non-purified eugenol and purified eugenol exhibit differences in their content. While non-purified eugenol is a mixture of several bioactive molecules, the purified eugenol is the isolated molecule from those extracts. In this study, non-purified eugenol comprised extracts obtained from natural sources, such as clove oil, clove extracts, and powdered cloves [29,30,93–98]. Different therapeutical

activity between these extracts may be related to the synergistic, additive, or unknown effects of their compounds [99]. Clove extracts, for instance, contain significant amounts of eugenol beside other compounds, such as eugenyl acetate and β-caryophyllene [100]. The isolation of compounds from plant material such as eugenol is crucial to lead discovery and drug screening. Accordingly, it is possible to determine their therapeutic function specifically [101]. Due to its separation and purification, it was possible to identify the eugenol role as an antidiabetic, antioxidant, and anti-inflammatory agent whose potential to abrogate oxidative stress is well established [66].

Moreover, low doses of eugenol induced significant responses in the insulin levels, body weight, and antioxidant activity. Of note, most part of the experiments tested the purified eugenol at low concentrations [32,58,65,66]. Once purified eugenol exhibits high potency, low doses are necessary to treat diabetes in murine. By contrast, studies evaluating non-purified eugenol administered extract at high concentrations [29,30,93–96,98], probably to achieve the concentration desirable of eugenol.

Finally, the type of diabetes influenced two variables in murine models. While type 2 diabetic animals showed a reduction in their glucose levels after eugenol treatment, the latter mitigated the liver damage in type 1 diabetic rodents. In the first case, eugenol was capable to activate the AMPK-GLUT4 pathway because type 2 diabetic animals have active pancreatic β-cells, which were responsive to the treatment and consequently produced insulin. Differently, in type 1 diabetic animals, the diabetogenic drugs STZ and alloxan destroyed their pancreatic β-cells, inactivating this pathway [102]. STZ is an antibiotic agent analog of N-acetylglucosamine, which is transported into pancreatic β-cells by GLUT2 and causes β-cell toxicity, generating free radicals and DNA damages [103]. Alloxan, in turn, is a pyrimidine nucleic acid that induces ROS formation and selectively inhibits glucose-induced insulin secretion through specific inhibition of glucokinase, the glucose sensor of pancreatic β-cells [102,104]. Unfortunately, none of the currently used medications reverse ongoing failure of β-cell function [29]. In this meta-analytical

review, beneficial effect of eugenol on liver damage was stronger in type 1 diabetic animals. Of note, only one study reported data on liver damage in type 2 diabetic animals [29], whereas other three studies evaluated the same parameters in type 1 diabetic rodents [58,59,95]. Thus, the number of studies evaluating this variable in type 1 diabetes may have influenced this finding. From those studies, two of them evaluated the effect of non-purified eugenol at 20 g/Kg [29] and 75 mg [95], and the others evaluated the purified eugenol at 2.5 mg [58] and 80 mg [59]. Despite the differences in the methodology of those studies, the mixed-model effect detected the influence of the type of diabetes on this variable. This fact might be better explored in future studies.

4.2. Limitations

In this review, the eligible studies show many differences in methodology such as various animal models used, different methods of inducing diabetes, and treatment (such as pure eugenol or not, and treatment time). It turns difficult to analyze the influence of each methodological parameter properly. For instance, a limitation of our review is that the studies were grouped into two categories of administered concentration since the intake range was very wide between groups. This might prevent definitive conclusions about the effect and the ideal dose to be administered. Even though, systematic reviews are considered high-level studies that allow individual assessment of studies in a blind manner, using specific tools [105]. Such characteristics lead to a more inclusive and reliable approach, providing a broad understanding of the included studies.

5. Conclusions and perspectives

To the best of knowledge, this meta-analysis provides the first compilation of information on the use of eugenol as a therapeutic compound against hyperglycemia. We have summarized the effects of eugenol by highlighting the key points of its effect on diabetes and also

complications associated with it. Undoubtedly, the decrease in glycemic levels by eugenol has been demonstrated through the inhibition of carbohydrate-metabolizing enzymes, and the possible inhibition of glucose production by the liver and the glucose uptake by skeletal muscle cells. The increase in antioxidant defense markers and the decrease in oxidative damage markers showed that eugenol could have potent effects on diabetic oxidative stress, restoring redox status and slowing the disease progression. As a result, eugenol also appears to regulate lipid profile and has a protective effect on liver and kidney tissues and, consequently, modulates body weight. Eugenol is probably more effective in its purified form than in mixtures such as extracts, clove oil, and powdered cloves. Nevertheless, studies evaluating the potential of eugenol as a complementary therapy to insulin treatment in type 1 diabetes or in association with other known antidiabetic drugs in type 2 diabetes could produce better results, leading to reduction of the side effects of current therapies. Thus, we believe that our findings may provide a baseline for further preclinical and clinical studies with important implications for human health.

Declaration of competing interest

No conflict of interest to declare.

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7. Supplementary Tables

Supplementary Table 1. Fail-safe numbers of eugenol meta-analysis.

Variables	Number of comparisons (*k*)	Rosenthal´s method (N = 5*k* + 10)
Liver damage	17	334.9
Glucose levels	42	1,103.5
Lipid profile	23	175.7
Oxidative damage	41	290.6
Kidney damage	31	253.4
Body weight	18	153.0
Antioxidant defense	67	675.7
Insulin levels	8	66.5
Carbohydrates-metabolizing enzymes	8	232.6

Supplementary Table 2. Summary of the results from mixed-effect model of meta-analysis showing the influence of methodological moderators on parameters.

Moderators	Influence[#]	Non-influence	NVG
Type of diabetes	Glucose levels Liver damage	Insulin levels (Q_B = 0.32; p = 0.57) Carbohydrates-metabolizing enzymes (Q_B = 0.45; p = 0.50) Body weight (Q_B = 2.20; p = 0.13) Antioxidant defense (Q_B = 0.46; p = 0.49) Oxidative damage (Q_B = 2.25.; p = 0.13) Lipid profile (Q_B = 2.86; p = 0.09)	Kidney damage[***]
Eugenol source	Liver damage Kidney damage Antioxidant defense Body weight Lipid profile	Glucose levels (Q_B = 1.90; p = 0.16) Carbohydrates-metabolizing enzymes (Q_B = 0.45; p = 0.50) Oxidative damage (Q_B = 1.57; p = 0.21)	Insulin levels[*]
Concentration administered	Body weight Antioxidant defense Insulin levels	Liver damage (Q_B = 0.69; p = 0.40) Glucose levels (Q_B = 0.44; p = 0.50) Lipid profile (Q_B = 0.26; p = 0.60) Oxidative damage (Q_B = 1.57; p = 0.21) Kidney damage (Q_B = 0.64; p = 0.42)	Carbohydrates-metabolizing enzymes[**]

Influence: methodological moderators influenced variables ($p < 0.05$); non-influence: methodological moderators did not influence variables ($p > 0.05$). [#]Detailed results are shown in the Fig. 3. NVG: There were not enough valid groups to perform the mixed-effect model because: there are available data of non-purified eugenol[*], concentrations > 10 mg Kg^{-1}[**], and type 1 diabetes[***] only.

3 CONCLUSÃO GERAL

Os resultados desta dissertação indicam que o eugenol é um composto com potencial efeito positivo no tratamento do diabetes. Com base nos resultados da meta-análise, demonstramos que o eugenol possui um efeito negativo sobre a hiperglicemia diabética. Os efeitos positivos na defesa antioxidante e efeitos negativos nos marcadores de dano oxidativo mostram o potencial do eugenol como aliado contra o estresse oxidativo causado pelo diabetes, restaurando o status redox e retardando a progressão da doença. Devido a esses efeitos, o eugenol também parece regular o perfil lipídico e ter um efeito protetor nos tecidos hepáticos e renais e, consequentemente, modular o peso corporal. Além disso, mesmo o eugenol sendo o principal composto bioativo do cravo, a administração do eugenol puro é mais eficaz no tratamento, quando comparada ao uso de extratos, óleos essenciais ou do cravo triturado. Portanto, mais estudos são necessários para se compreender melhor o mecanismo de ação do eugenol no tratamento do diabetes, seja em modelos animais ou em estudos clínicos. Nossos próximos estudos se concentrarão em estudar o efeito do eugenol no sistema reprodutor masculino e também do uso do eugenol como terapia complementar a insulina e/ou drogas antidiabéticas em animais induzidos experimentalmente ao diabetes.