Article published online: 2022-09-28

A Systematic Review of the Potential Effects of Propolis Extracts on Experimentally-induced Diabetes

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Key words
propolis, flavonoids, diabetes, bee glue

received May 2, 2022
accepted after revision July 20, 2022
published online September 28, 2022

Bibliography
Planta Med 2023; 89: 236–244
DOI 10.1055/a-1910-3505
ISSN 0032-0943
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Georg Thieme Verlag KG, Rüdigerstraße 14,
70469 Stuttgart, Germany

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Supplementary material is available under https://doi.org/10.1055/a-1910-3505

ABSTRACT

Oxidative stress (OS) is involved in the development of diabetes mellitus (DM) and its complications. Thus, OS reduction may be an important strategy for DM therapy. Propolis is bee resins with high antioxidant activity and is used in the treatment of different diseases, including DM. Therefore, in this systematic review, we evaluated the impact of propolis administration in diabetic animals. We used the PRISMA strategy to collect preclinical studies published in English up to November 2021 in three databases (PubMed/Medline, Scopus, and Web of Science). We used the SYRCLE tool to analyze the risk of methodological bias. Our primary search returned 198 studies, of which 14 were considered eligible to be included in this review. The administration of propolis induced a hypoglycemic effect in the treated animals, which is probably due to the reduction of OS. The animals showed restoration of endogenous antioxidant defenses and reduced levels of markers for OS. The administration of propolis resulted in improvement in the lipid profile of treated animals. Our risk of bias assessment showed a methodological quality score of less than 30 % due to a lack of randomization, blinding, and proper allocation of animals. Heterogeneity in treatments, lack of results, and use of non-standard extracts are limitations in our data analysis. Despite these limitations, propolis induced a significant hypoglycemic effect in diabetic animals when compared to untreated controls. This effect was associated with a reduction in OS, a process mediated by ROS neutralization and restoration of endogenous antioxidant defenses.

Introduction

Diabetes mellitus (DM) is a chronic endocrine disease characterized by constant hyperglycemia that results from a deficiency in insulin production (type 1 DM) or sensitivity (type 2 DM) [1]. Currently, over 536.6 million individuals worldwide have diabetes and approximately 6.7 million deaths are directly related to this disease in 2021 [2]. Although diabetes affects millions of individuals, the causes that lead to the development of the disease are still not fully understood [3].

The overproduction of reactive oxygen species (ROS) and oxidative stress (OS) are identified as key factors in the development of DM and diabetic complications [4–6]. Hyperglycemia increases the flow of glucose through the polyol pathway and stimulates the

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ABBREVIATIONS

ApoA–I	apolipoprotein A–I
ApoB	apolipoprotein B
ATP	adenosine triphosphate
CAT	catalase
DM	diabetes mellitus
DNA	deoxyribonucleic acid
GRAS	generally recognized as safe
GSH	glutathione
GPx	glutathione peroxidase
HDL	high-density lipoprotein
Hmox1	heme oxygenase 1 gene
LDL	low-density lipoprotein
MDA	malondialdehyde
MeSH	medical subject headings
mRNA	messenger ribonucleic acid
Nqo1	NAD(P)H quinone dehydrogenase 1 gene
Nrf2	nuclear factor erythroid 2-related factor 2
OS	oxidative stress
PRISMA	preferred reporting items for systematic reviews and meta-analysis
PROSPERO	international prospective registry of systematic reviews
PUFA	polyunsaturated fatty acids
RNS	reactive nitrogen species
ROS	reactive oxygen species
SOD	superoxide dismutase
STZ	streptozotocin
SYRCLE	systematic review center for laboratory animal experimentation
TBARS	thiobarbituric acid reactive substances

formation of advanced glycation end products (AGEs), molecules that promote the overproduction of ROS [7–9]. In the pancreas, ROS induce a decrease or inhibition of insulin secretion through beta-cell destruction and DNA alterations [5, 6]. Despite the direct influence of ROS and OS on the pathogenesis of DM, none of the current drugs have reducing mechanisms that can directly inhibit them. Therefore, the consumption of antioxidant agents may represent an important therapeutic strategy in the management of DM [4, 9–11].

Bee products are considered sources of natural antioxidants capable of neutralizing ROS and reducing the effects of OS underlying the pathogenesis of numerous diseases [10, 12–14]. Propolis is a chemically diverse resinous product produced by *Apis mellifera* or stingless bees that is made of plant exudates as well as wax, pollen, and honey [11, 12]. Phenolic compounds are mainly responsible for the ability to scavenge free radicals and other biological properties performed by propolis [12–16]. Over the years, several studies have highlighted the biological properties of bee resins, and their applicability in the management of different diseases [17–19]. Therefore, the administration of propolis in the treatment of patients with DM may represent a therapeutic strategy, considering its high antioxidant activity and other biological properties [8, 9, 14, 19].

Despite its potential effect as an antidiabetic agent, studies in experimental animal models provide the empirical basis for determining the efficacy, safety, and applicability of propolis extracts in human clinical studies [20, 21]. However, as the results of preclinical studies often originate from relatively small experiments with heterogeneous methodologies, they may not always be applicable in a translational context for human health [20]. Therefore, systematic reviews of preclinical studies can provide us with reliable evidence of whether or not propolis extracts and their derivatives have benefits in the treatment of DM and support their application in clinical trials [21]. Thus, we carried out a systematic review to determine the relevance and impact of propolis administration on experimentally induced diabetes in animal models, mainly on outcomes related to redox balance, glycemic, and lipidic profiles.

Results

Prisma-guided studies selection

Searches in the three databases returned a total of 198 studies (PubMed/Medline n = 36; Scopus n = 112; Web of Science n = 50), from which 9 literature reviews (review articles) were directly excluded. The other studies were imported into the Mendeley reference manager, and 76 duplicates were removed by the "Check for duplicates" tool. The titles and abstracts of 109 articles were read, of which 62 were excluded for not falling into the scope of the systematic review. Forty-seven studies were considered eligible for full-text analysis, but 9 of them could not be retrieved. Hence, 38 articles were read in full and screened for the study criteria, of which 14 articles were selected for this systematic review. The secondary search from the reference lists of selected articles did not return any relevant studies. A detailed flowchart of the search strategy is shown in ▶ **Fig. 1**. The search filters used in the databases are available in Supplementary **Table 1S**, Supporting Information.

Characteristics of selected studies

There was heterogeneity regarding the origin of the selected studies, and articles from 11 countries were retrieved. With 14% of publications originating from China, 14% from Malaysia, 14% from Nigeria, 7.25% from Egypt, 7.25% from Indonesia, 7.25% from Japan, 7.25% from Morocco, 7.25% from Mexico, 7.25% from Saudi Arabia, 7.25% from Taiwan, and 7.25% from Turkey.

The administration of streptozotocin was the DM induction method in 78.57% of the selected studies, followed by the injection of alloxan (21.43%). The main route of drug administration was intraperitoneal (71.42%), followed by the intravenous route (28.58%). The data described above can be viewed in detail in Supplementary **Table 2S**, Supporting Information.

Characteristics of animal models

Sprague-Dawley rats (49.90%) and Wistar rats (35.90%) were the main models chosen for the induction of DM, respectively. CD1 mice were used in 14.20% of the studies. Male animals were used

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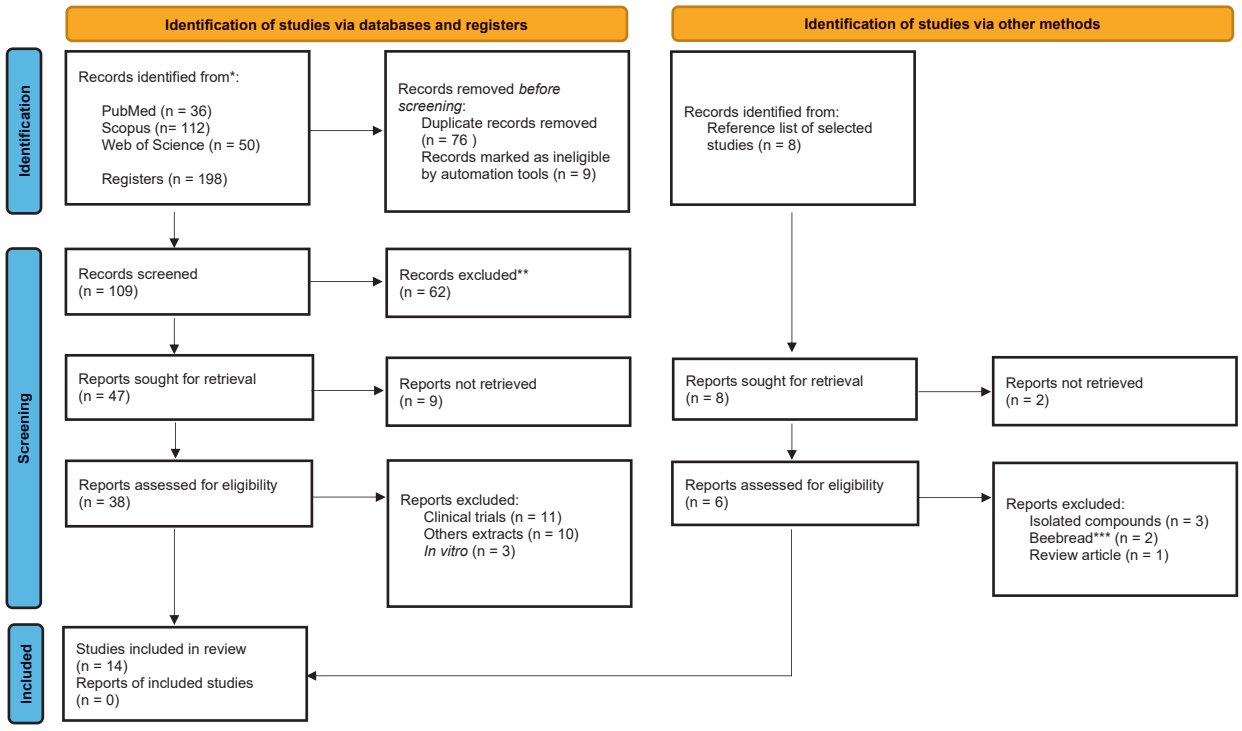

*Consider, if feasible to do so, reporting the number of records identified from each database or register searched (rather than the total number across all databases/registers).
**If automation tools were used, indicate how many records were excluded by a human and how many were excluded by automation tools.
***A mixture of pollen and nectar

From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71. For more information, visit: http://www.prisma-statement.org/

▶ **Fig. 1** PRISMA flowchart describing the results of the searches in the databases and the reference list of the selected studies. Based on the PRISMA statement "Preferred Reporting Items for Systematic Reviews and Meta-analyses" (http://www.prisma-statement.org/).

in 85.80% of the publications and females in 7.10%. In 7.10% of the articles, the sex of the animals is not reported.

The average weight of animals was 260 g for rats and 37.50 g for mice. The age of the animals was omitted in 57.10% of the studies; 28.40% described the age in days (mean of 60 days), and 14.50% of them considered the animals to be adults (criterion established by the authors). Detailed characteristics of the animal models are presented in Supplementary **Table 3S**, Supporting Information.

Characteristics of propolis

The most frequent origin of the propolis samples used was China (14%), Nigeria (14%), and Malaysia (14%). The other bee resins were of heterogeneous origin, with 7.25% from Egypt, 7.25% from Indonesia, 7.25% from Brazil, 7.25% from Morocco, 7.25% from Mexico, 7.25% from Saudi Arabia, 7.25% from Taiwan, and 7.25% from Turkey.

Propolis extracts were administered mostly orally (92.8%) and less frequently via the intragastrical route (7.2%) at doses ranging from 10 to 919.5 mg/kg. The most common doses were 300 mg/kg (50%) and 200 mg/kg (35.7%). The effectiveness of propolis administration was dose-dependent, and there were no reports of toxicity. The shortest exposure time of the animals to propolis extracts was 7 days, and the maximum was 70 days. All results described above can be viewed in detail in Supplementary **Table 4S**, Supporting Information.

Identified compounds

The propolis samples that were chemically identified showed great heterogeneity in their composition. In 57.2% of the studies, there was no chemical characterization of the extract, indicating that animals were exposed to crude extracts whose chemical composition was unknown. Alarmingly, only 42.8% of the authors performed the identification of compounds by chromatographic methods. ▶ **Table 1** shows the major compounds tentatively identified in the samples of the selected studies.

Measured Outcomes

Measured primary outcomes

The glycemic index was measured in 92.8% of the studies. The animals were characterized as diabetic when they had blood glucose levels above 11.1 mmol/L or greater than 200 mg/dL. The administration of propolis reduced the glycemic levels of the treated an-

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▶ **Table 1** Major compounds identified by selected studies and their effects on animal models.

Authors	Propolis origin	Chemical profile	Animal/Dosage*/Route	Effects
Matsushige et al. 1996	Brazil	Clerodane diterpenoid Quercetin	Sprague-Dawley rats **Dosage:** 200 mg/kg **Route:** orally	↓ Blood glucose levels
Usman et al. 2017	Malaysia	Glucuronic acid derivatives Ellagic acid Gallic acid derivatives	Sprague-Dawley rats **Dosage:** 300 and 600 mg/kg **Route:** orally	↓ Blood glucose levels ↓ MDA levels
Chen et al. 2018	Taiwan	Propolin (D, F, C, H, and G)	Sprague-Dawley rats **Dosage:** 183.9 and 919.5 mg/kg **Route:** oral gavage	↓ Blood glucose levels ↓ TBARS levels ↓ LDL levels ↑ HDL levels
Yañes et al. 2018	Mexico	Naringin Quercetin Luteolin Kaempferol	CD1 mices **Dosage:** 300 mg/kg **Route:** orally	↓ Blood glucose levels ↑ SOD, CAT, GPx levels
Hegazy et al. 2020	Egypt	2-[3,4-(Methylenedioxy) Phenyl]-1-Cyclopentanone 3-(2h)-Pyridazinone, 4,5-Dihydro-4-(4-Methoxyphenyl) 7-Methoxy-3,6-Dimethyl-2-Tetralone 2′-Hydroxy-2,3,4′,6′-Tetramethoxychalcone	Wistar rats **Dosage:** 300 mg/kg **Route:** oral gavage	↓ Blood glucose levels
Taleb et al. 2020	Turkey	Chrysin Caffeic acid phenyl ester	Wistar rats **Dosage:** extracts 15 and 30% **Route:** orally	↓ Blood glucose levels

Hypoglycemic, antioxidant and non-toxic activities of the extracts are noted. CAT – catalase; GPx – glutathione peroxidase; SOD – superoxide dismutase; TBARS – thiobarbituric acid reactive substances; MDA – malondialdehyde; LDL low-density lipoprotein; HDL – high-density lipoprotein. * Dosage of propolis extracts

imals compared to the untreated ones. The authors pointed to the decrease of OS as responsible for the hypoglycemic effect.

The main molecules measured to describe changes in the redox balance in the animals' bodies were MDA (malondialdehyde) and TBARS (thiobarbituric acid reactive substances). The antioxidant potential of propolis is related to the occurrence of phenolic compounds, whose chemical structure allows for the donation of electrons to unstable molecules (ROS or RNS), thereby reducing oxidative damage. Decreased OS was also associated with increases in endogenous antioxidant enzyme levels. Antioxidant enzymes such as SOD (superoxide dismutase), CAT (catalase), GPx (glutathione peroxidase), and the tripeptide GSH (glutathione) showed a significant increase in animals treated with propolis compared to those not treated.

Measured secondary outcomes

The administration of propolis also promoted changes in the lipid profile of the treated animals. Overall, the extracts induced an increase in HDL cholesterol levels and a decrease in LDL cholesterol levels. All results described above can be seen in Supplementary **Table 5S**, Supporting Information.

Risk of methodological bias analyzed by the Syrcle's tool

In general, all studies analyzed presented a quality score of less than 30% based on the SYRCLE tool due to lack of relevant information for methodological development (inadequate georeferencing of propolis and lack of chemical characterization) and neglect of criteria such as randomization, allocation of animals and blinding of examiners. However, variables such as animal weight, propolis administration routes, DM induction method, and exposure time were described in 100% of the studies (▶ **Fig. 2**).

Discussion

In this review, most studies that investigated the effects of propolis on DM were Chinese, Malaysian, and Nigerian. According to the International Diabetes Federation [2], these countries have a high prevalence of diabetes. China and Malaysia are in the western Pacific, a region whose growth in the number of DM cases will exceed 27% by the year 2045. Moreover, a 134% increase in the number of cases is expected for the African continent until that same year. Asian and African countries have been exploring the use of natural products for several centuries, particularly herbal infusions and medicinal extracts [22, 23]. We also note that all these studies explored local bee resins in their raw

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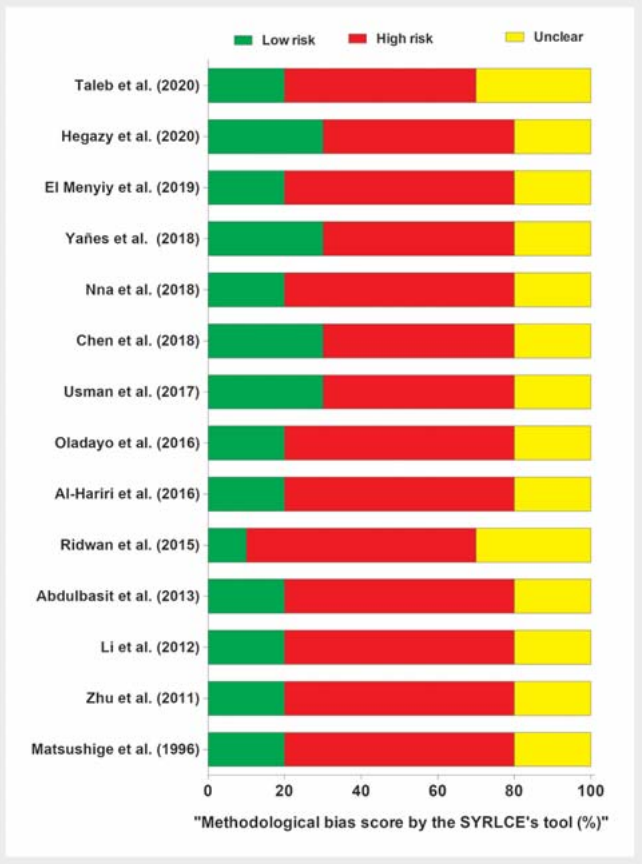

▶ **Fig. 2** Risk of methodological bias analysis of selected studies using SYRCLE's tool.

form and that isolated compounds from propolis were not evaluated.

Despite the alarming situation observed with the global increase in DM cases, intervention in humans requires robust evidence from preclinical *in vivo* trials to ensure the safety and efficacy of the active compound [20]. The literature indicates rodent models as a species of choice to mimic the diabetic effects that would be observed in humans, as they present high similarity with our DNA (85% in coding regions) and are easy to handle [24, 25]. For DM induction, intraperitoneal injection with 60 mg/kg streptozotocin (STZ) was the main method used by the authors, STZ (2-deoxy-2-(3-(methyl-3-nitrosoureido)-D-glucopyranose). The toxicity of STZ is dependent on the DNA alkylating activity of its methylnitrosourea moiety [26, 27]. Diabetic effects were also induced by intraperitoneal injections of alloxan (2,4,5,6-tetraoxypyrimidine; 5,6-dioxiacyl), whose dosages were greater than 100 mg/kg. Alloxan acts by selectively inhibiting glucose-induced insulin secretion through specific inhibition of glucokinase. According to Lenzen (2008) [27], streptozotocin is the agent of choice for DM induction in animals due to its chemical characteristics and high stability. Alloxan, on the other hand, is an excellent compound for ROS-mediated beta-cell toxicity models, although its effects are reported to be more severe in rat beta cells than in humans [26].

The oral administration of propolis effectively controlled the glycemic levels of the animals. These findings are intriguing since the flavonoids present in propolis (and in any other food of natural origin) have low bioavailability (10% or less), suggesting they have strong antidiabetic activity [28, 29]. Those effects were dose-dependent and observed at all tested doses, with optimal effectiveness between 200 and 300 mg/kg. Higher dosages did not show significantly greater benefits.

The chemical composition of propolis is variable depending on environmental characteristics and extraction methods, which may have affected the final biological response [30, 31]. Therefore, the chemical characterization of the selected samples is necessary to elucidate which molecules are responsible (alone or synergistically) for the antidiabetic effects and/or to purify through chromatographic methods by the most active ones, if applicable [15, 32, 33].

Even though propolis samples were not chemically characterized in most studies, the main antidiabetic agents in propolis are flavonoids. Based on the literature, quercetin, naringin, luteolin, kaempferol, and chrysin have hypoglycemic effects by inducing insulin secretion, increasing the sensitivity of skeletal muscles to glucose, and selectively inhibiting α-amylase and α-glucosidase, although other metabolic pathways are also involved [11, 29, 34]. The effective propolis doses (200 to 300 mg/kg) are not toxic, according to the Food and Drug Administration, because propolis is generally recognized as safe (GRAS) [35]. These doses are lower than those used in the standard treatment of type 2 DM with the drug metformin, whose doses vary from 500 mg/kg twice daily to 800 mg/kg daily [36].

In our review, the hypoglycemic effect observed in propolis was due to the neutralization of ROS in the pancreatic tissue and the increase of endogenous antioxidant defenses. Hyperglycemia activates numerous metabolic pathways that culminate in the generation of ROS, which can induce DNA changes, promote peroxidation of the phospholipid bilayer, and lead to ATP deficit [5, 6, 8, 9]. Altogether, these mechanisms promote the necrosis of pancreatic β cells, resulting in insulin deficiency [4, 5]. According to Newsholme et al. (2016) [6], it is extremely difficult to measure changes in ROS levels in the body, as reactive species have an extremely short half-life in biological fluids, cells, and tissues.

Consequently, researchers have developed other techniques to determine the redox state, and these usually involve the assessment of stable byproducts of OS in the blood. In this review, MDA was the main marker for OS. In biological systems, this molecule is a byproduct of the lipid peroxidation of cell membranes as a consequence of the reaction of polyunsaturated fatty acids (PUFAs) and radical species [37, 38]. Compared to ROS, MDA has a relatively long half-life (minutes-hours) and an uncharged structure, making it a potentially more destructive compound [38]. Induced DM promoted alterations in the lipid peroxidation rate, as demonstrated by the plasma levels of MDA, which were reduced following the administration of propolis. Although the main antioxidant mechanism of propolis is the donation of electrons to ROS with their consequent stabilization, the studies also reported a reduction of lipid peroxidation, with the restoration of the body's endogenous enzyme antioxidant system.

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The enzymes SOD, CAT, GPx, and the tripeptide GSH had their plasma levels increased in all studies that examined these variables. There were no reports of the possible causes that led to this result; however, the activation of the nuclear factor erythroid 2-related factor 2 (*Nrf2*) was pointed out in previous studies. *Nrf2* is a transcription factor that acts as the main regulator of the antioxidant response. In situations of OS, it migrates into the cell nucleus and activates genes involved in the expression of endogenous antioxidant enzymes and other ROS scavenging mechanisms [39, 40]. Hotta et al. (2020) [40] demonstrated that treatment with Brazilian red propolis increased the mRNA levels of *Nrf2, Nqo1, Hmox1* genes responsible for the activation of endogenous antioxidant defenses. While it may not be pertinent to extrapolate these findings to the propolis types described in this review, the data suggest the *Nrf2* activation pathway is likely to be involved.

Lastly, the administration of propolis induced an increase in HDL and a decrease in LDL levels in all the studies that examined these variables. Yet, further research is needed to elucidate the metabolic pathways and possible molecular targets involved in both processes. A possible route of action would be to control the expression of apolipoproteins, protein subunits responsible for the stabilization and transport of cholesterol molecules. In *in vitro* assays, quercetin and isoquercitrin positively modulated the expression of apolipoprotein A–I (apoA–I), a subunit presents in HDL [41]. On the other hand, animals treated with naringenin showed a 36% reduction in the secretion of apolipoprotein B (apoB), a protein is related to LDL synthesis [42]. The compounds occurring in the propolis samples described in our review are likely to have similar effects in controlling the plasma levels of high and low-density lipoproteins. The changes observed in the lipid profile of animals provide parameters to explore the effects of propolis administration on other diseases, especially cardiovascular disorders that are directly linked to fat deposition in the blood vessel wall [43, 44].

The methodological consistency of preclinical studies must be considered when examining the quality of evidence to support future clinical trials [45]. Surprisingly, none of the analyzed studies met all the methodological criteria proposed by the SYRCLE (▶ **Fig. 2**), presenting variable scores without chronological influence (year of publication). This result indicates that the reporting bias was systematically reproduced through the mechanistic research process, without interpretations of possible sources of bias. The main neglected aspects were randomization, precise georeferencing of the origin of propolis, animal allocation, randomization, and chemical composition of propolis [45–47]. Finally, we make it clear that our objective was not to confront the current results, nor to devalue them, but to verify the possible sources of current methodological bias and, from such notes, provide support for data consistency and reproducibility.

Although our systematic review represents a proposal to critically analyze the evidence on the applicability of propolis extracts in the treatment of diabetes, our interpretations of the results must consider some limitations. Our searches were limited to databases and reference lists of selected articles. Thus, the search strategy adopted may have excluded relevant studies [21, 48]. Some studies did not present all the evaluated outcomes of interest. Therefore, we must consider the absence of results as a limitation, as it was not possible to assess the current evidence in its entirety.

The lack of standardization and chemical characterization of the extracts is also a limiting factor. The conditions adopted during extraction, storage, and preparation affect the physicochemical characteristics of these formulations [49, 50]. Therefore, our analysis of extracts without standardization could be controversial if variations in these conditions were adopted. The biological properties of bee resins result from the association between different compounds. However, environmental variations can change its chemical composition [31, 33]. Thus, the analysis of studies that did not chemically characterize propolis may represent a limitation, as changes in the collection time of bee resins influence the effectiveness of their biological properties.

In conclusion, propolis induced a significant hypoglycemic effect in diabetic animals when compared to untreated controls. This effect was associated with a reduction in OS, a process mediated by ROS neutralization and restoration of endogenous antioxidant defenses. Propolis reestablished plasma levels of HDL and reduced those of LDL, possibly by modulating the transcription of apolipoproteins. We also emphasize the need to review some methodological aspects to mitigate the sources of bias in preclinical approaches and ensure reproducibility in future studies, especially of criteria such as randomization, blinding, and characterization of propolis samples.

Methods

Guiding question and search strategy

The PICO strategy was adopted to structure the research question, thus ensuring that the relevant components of the question are well defined. According to Eriksen and Frandsen (2018) [51], the PICO structure is articulated to meet all four parts of its "anatomy": P- population (diabetic animals); I- intervention or exposure (administration of propolis); C- comparator (untreated animals); O- outcomes (glycemic indices and oxidative parameters). Therefore, this systematic review was designed to answer the following guiding question: Is the administration of propolis effective in controlling hyperglycemia and OS in animals with experimentally induced diabetes when compared to untreated diabetic animals?

To answer the guiding question, primary studies were selected based on the PRISMA strategy – Preferred Reporting Items for Systematic Reviews and Meta-analysis [48]. Relevant studies were selected from three databases, namely: PubMed/Medline, Scopus, and Web of Science. We associated MeSH terms found in Medical Subject Headings (MeSH), standardized descriptors specific to each database, and Boolean operators (AND/OR/NOT) to build the search filters. We structured them into three levels of research: (i) biological condition (diabetes), (ii) intervention (propolis), and (iii) study groups (animal models). The complete search strategy can be seen in Supplementary **Table 1S**, Supporting Information. This systematic review was registered in the International Prospective Registry of Systematic Reviews – PROSPERO (registration number: CRD42021290848).

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Studies selection

Literature searches were structured into two levels of information (primary and secondary) to ensure access to the greatest number of relevant studies. Initially, the studies were identified in the three electronic databases. In the Scopus platform, the descriptor NOT INDEX MEDLINE was associated with the search terms to ensure the removal of duplicate studies from PubMed/Medline. Identified primary studies were managed in the Mendeley Reference Management Program (Mendeley, London, Westminster, UK) and duplicates were removed using the "Check for Duplicates" tool. Retrieved studies were then screened for eligibility. Studies falling out of the scope of this review were excluded. In the secondary search, the reference lists of relevant articles that were selected in the primary search were checked manually to identify possible additional studies. These search strategies are described in the PRISMA flowchart [48].

The other studies were accessed in full and included in the eligibility analysis, in which well-defined inclusion and exclusion criteria were applied. Studies that addressed the effects of propolis administration on diabetic animals were considered relevant. The following studies were excluded: (i) not available in full; (ii) studies that were not written in English; (iii) studies with other bee products (e.g., royal jelly or honey); (iv) secondary studies (e.g., letters to the editor, conference abstracts, commentaries, notes, and books); (v) studies that did not have at least one control group; (vi) studies of diabetic disorders (retinopathies, nephropathies, or diabetic wounds). No chronological limits were applied for the selection of eligible studies.

Two researchers (Cunha, GA, and Carlstrom, PF) independently completed the screening for eligibility and study selection. Any disagreements between the examiners were resolved by consulting with a third examiner (Rosalen, PL).

Data extraction

After study selection, the data were structured in graphs and tables to facilitate the visualization and identification of outcomes. Two examiners (Cunha, GA, and Carlstrom, PF) evaluated the survey data, and differences were resolved by consensus in consultation with the third examiner (Rosalen, PL). The following descriptive levels were adopted:

1. Study characteristics: year, author, country of origin, DM induction method, study groups;
2. Characteristics of the animal model(s): age, species, lineage, body weight, sex;
3. Characteristics of propolis: origin, chemical profile, the form of administration, dosage, period of intervention, and;
4. Measured outcomes: oxidative parameters, glycemic levels, and lipid profile.

Bias risk assessment

The risk of bias was determined using the SYRCLE risk of bias tool for animal studies. This tool was developed following the Cochrane Risk of Bias (ROB) tool, with adjustments for specific aspects of bias with a relevant impact on intervention animal studies. The SYRCLE tool is stratified into ten topics related to potential sources of bias, such as (i) selection, (ii) performance, (iii) detection, (iv) friction, (v) reporting, and (vi) additional sources of bias not covered by other domains [52]. Based on the SYRCLE criteria, the risk of bias was categorized as: (i) High, (ii) Low, or (iii) Unclear. The overall and individual result obtained with the SYRCLE strategy was graphically expressed using GraphPad Prism version 5.0.

Supporting Information

The information taken from the studies is summarized in tables available in the Supporting Information.

Contributors' Statement

Study design: G. A. Cunha, P. F. Carlstrom, P. L. Rosalen, M. Ikegaki, S. M. Alencar, M. Franchin. Data collection: G. A. Cunha, P. F. Carlstrom, P. L. Rosalen. Analysis and interpretation of the data: G. A. Cunha, P. F. Carlstrom, P. L. Rosalen, M. Ikegaki, S. M. Alencar, M. Franchin. Drafting the manuscript: G. A. Cunha, P. F. Carlstrom, P. L. Rosalen, M. Ikegaki, S. M. Alencar, M. Franchin. Critical review of the manuscript: P. L. Rosalen, M. Ikegaki, S. M. Alencar, M. Franchin.

Acknowledgements

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brazil (CAPES) – Finance Code 001.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

[1] World Health Organization. Diabetes. 2021. Accessed June 9, 2021 at: https://www.who.int/health-topics/diabetes#tab=tab_1

[2] International Diabetes Federation. IDF Diabetes Atlas 2021 10th. International Diabetes Federation 2021. Accessed December 19, 2021 at: https://diabetesatlas.org/atlas/tenth-edition/

[3] Kharroubi AT, Darwish HM. Diabetes mellitus: The epidemic of the century. World J Diabetes 2015; 6: 850. doi:10.4239/wjd.v6.i6.850

[4] Deng L, Du C, Song P, Chen T, Rui S, Armstrong DG, Deng W. The role of oxidative stress and antioxidants in diabetic wound healing. Oxid Med Cell Longev 2021; 2021: 8852759. doi:10.1155/2021/8852759

[5] Luc K, Schramm-Luc A, Guzik TJ, Mikolajczyk TP. Oxidative stress and inflammatory markers in prediabetes and diabetes. J Physiol Pharmacol 2019; 70: 809–824. doi:10.26402/jpp.2019.6.01

[6] Newsholme P, Cruzat VF, Keane KN, Carlessi R, Bittencourt PIH. Molecular mechanisms of ROS production and oxidative stress in diabetes. Biochem J 2016; 473: 4527–4550. doi:10.1042/BCJ20160503C

[7] Kaneto H, Katakami N, Matsuhisa M, Matsuoka TA. Role of reactive oxygen species in the progression of type 2 diabetes and atherosclerosis. Mediators Inflamm 2010; 2010: 453892. doi:10.1155/2010/453892

[8] Grabež M, Škrbić R, Stojiljković MP, Vučić V, Rudić Grujić V, Jakovljević V, Djuric DM, Suručić R, Šavikin K, Bigović D, Vasiljević N. A prospective, randomized, double-blind, placebo-controlled trial of polyphenols on the outcomes of inflammatory factors and oxidative stress in patients with type 2 diabetes mellitus. Rev Cardiovasc Med 2022; 23: 57. doi:10.31083/j.rcm2302057

[9] Domanico D, Fragiotta S, Cutini A, Carnevale C, Zompatori L, Vingolo E. Circulating levels of reactive oxygen species in patients with nonproliferative diabetic retinopathy and the influence of antioxidant supplementation: 6-Month follow-up. Indian J Ophthalmol 2015; 63: 9–14. doi:10.4103/0301-4738.151455

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[10] Nna VU, Abu Bakar AB, Md Lazin MRML, Mohamed M. Antioxidant, anti-inflammatory and synergistic anti-hyperglycemic effects of Malaysian propolis and metformin in streptozotocin–induced diabetic rats. Food Chem Toxicol 2018; 120: 305–320. doi:10.1016/j.fct.2018.07.028

[11] Babu PVA, Liu D, Gilbert ER. Recent advances in understanding the anti-diabetic actions of dietary flavonoids. J Nutr Biochem 2013; 24: 1777–1789. doi:10.1016/j.jnutbio.2013.06.003

[12] Kocot J, Kiełczykowska M, Luchowska-Kocot D, Kurzepa J, Musik I. Antioxidant potential of propolis, bee pollen, and royal jelly: Possible medical application. Oxid Med Cell Longev 2018; 2018: 7074209. doi:10.1155/2018/7074209

[13] Braakhuis A. Evidence on the health benefits of supplemental propolis. Nutrients 2019; 11: 2705. doi:10.3390/nu11112705

[14] Tiveron AP, Rosalen PL, Franchin M, Lacerda RCC, Bueno-Silva B, Benso B, Denny C, Ikegaki M, Alencar SM. Chemical characterization and antioxidant, antimicrobial, and anti-inflammatory activities of South Brazilian organic propolis. PLoS One 2016; 11: e0165588. doi:10.1371/journal.pone.0165588

[15] Silva BB, Rosalen PL, Cury JA, Ikegaki M, Souza VC, Esteves A, Alencar SM. Chemical composition and botanical origin of red propolis, a new type of Brazilian propolis. Evid Based Complement Alternat Med 2008; 5: 313–316. doi:10.1093/ecam/nem059

[16] Vieira de Morais D, Rosalen PL, Ikegaki M, Silva APS, Massarioli AP, Alencar SM. Active antioxidant phenolics from Brazilian red propolis: An optimization study for their recovery and identification by LC-ESI-QTOF-MS/MS. Antioxidants (Basel) 2021; 10: 297. doi:10.3390/antiox10020297

[17] Sforcin JM, Bankova V. Propolis: Is there a potential for the development of new drugs? J Ethnopharmacol 2011; 133: 253–260. doi:10.1016/j.jep.2010.10.032

[18] Silva SS, Mizokami SS, Fanti JR, Miranda MM, Kawakami NY, Teixeira FH, Araújo EJA, Panis C, Watanabe MAE, Sforcin JM, Pavanelli WR, Verri WA, Felipe I, Conchon-Costa I. Propolis reduces Leishmania amazonensis-induced inflammation in the liver of BALB/c mice. Parasitol Res 2016; 115: 1557–1566. doi:10.1007/s00436-015-4890-4

[19] Nani BD, Sardi JCO, Lazarini JG, Silva DR, Massariolli AP, Cunha TM, de Alencar SM, Franchin M, Rosalen PL. Anti-inflammatory and anti-*Candida* effects of Brazilian organic propolis, a promising source of bioactive molecules and functional food. J Agric Food Chem 2020; 68: 2861–2871. doi:10.1021/acs.jafc.8b07304

[20] Hooijmans CR, Ritskes-Hoitinga M. Progress in using systematic reviews of animal studies to improve translational research. PLoS Med 2013; 10: e1001482. doi:10.1371/journal.pmed.1001482

[21] Pereira RM, Greco GMZ, Moreira AM, Chagas PF, Caldas IS, Gonçalves R, Novaes RD. Applicability of plant-based products in the treatment of Trypanosoma cruzi and Trypanosoma brucei infections: A systematic review of preclinical *in vivo* evidence. Parasitology 2017; 144: 1275–1287. doi:10.1017/S0031182017000634

[22] Yuan H, Ma Q, Ye L, Piao G. The traditional medicine and modern medicine from natural products. Molecules 2016; 21: 559. doi:10.3390/molecules21050559

[23] Moroole MA, Materechera SA, Mbeng WO, Aremu AO. Medicinal plants used for contraception in South Africa: A review. J Ethnopharmacol 2019; 235: 19–27. doi:10.1016/j.jep.2019.02.002

[24] Barré-Sinoussi F, Montagutelli X. Animal models are essential to biological research: Issues and perspectives. Future Sci OA 2015; 1: FSO63. doi:10.4155/fso.15.63

[25] King AJF. The use of animal models in diabetes research. Br J Pharmacol 2012; 166: 877–894. doi:10.1111/j.1476-5381.2012.01911.x

[26] Szkudelski T. The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. Physiolog Res 2001; 50: 537–546

[27] Lenzen S. The mechanisms of alloxan- and streptozotocin-induced diabetes. Diabetologia 2008; 51: 216–226. doi:10.1007/s00125-007-0886-7

[28] Thilakarathna SH, Vasantha Rupasinghe HP. Flavonoid bioavailability and attempts for bioavailability enhancement. Nutrients 2013; 5: 3367–3387. doi:10.3390/nu5093367

[29] Hu M. Commentary: Bioavailability of flavonoids and polyphenols: Call to arms. Mol Pharm 2007; 4: 803–806. doi:10.1021/mp7001363

[30] Cavalaro RI, Cruz RGD, Dupont S, de Moura Bell JMLN, Vieira TMFS. *In vitro* and *in vivo* antioxidant properties of bioactive compounds from green propolis obtained by ultrasound-assisted extraction. Food Chem X 2019; 4: 100054. doi:10.1016/j.fochx.2019.100054

[31] Park YK, Alencar SM, Aguiar CL. Botanical origin and chemical composition of Brazilian propolis. J Agric Food Chem 2002; 50: 2502–2506. doi:10.1021/jf011432b

[32] Cavalaro RI, Fabricio LFF, Vieira TMFS. Ultrasound-assisted extraction of antioxidants from *Baccharis dracunculifolia* and green propolis. Processes 2020; 8: 1530. doi:10.3390/pr8121530

[33] Bueno-Silva B, Marsola A, Ikegaki M, Alencar SM, Rosalen PL. The effect of seasons on Brazilian red propolis and its botanical source: chemical composition and antibacterial activity. Nat Prod Res 2017; 31: 1318–1324. doi:10.1080/14786419.2016.1239088

[34] Al-Ishaq RK, Abotaleb M, Kubatka P, Kajo K, Büsselberg D. Flavonoids and their anti-diabetic effects: Cellular mechanisms and effects to improve blood sugar levels. Biomolecules 2019; 9: 430. doi:10.3390/biom9090430

[35] El-Guendouz S, Aazza S, Lyoussi B, Majdoub N, Bankova V, Popova M, Raposo S, Antunes MD, Miguel MG. Effect of poplar-type propolis on oxidative stability and rheological properties of O/W emulsions. Saudi Pharm J 2018; 26: 1073–1082. doi:10.1016/j.jsps.2018.05.017

[36] Mahabaleshwarkar R, DeSantis A. Metformin dosage patterns in type 2 diabetes patients in a real-world setting in the United States. Diab Res Clin Pract 2021; 172: 108531. doi:10.1016/j.diabres.2020.108531

[37] Jové M, Mota-Martorell N, Pamplona R, Pradas I, Martín-Gari M, Ayala V. The advanced lipoxidation end-product malondialdehyde-lysine in aging and longevity. Antioxidants (Basel) 2020; 9: 1132. doi:10.3390/antiox9111132

[38] Czerska M, Mikołajewska K, Zieliński M, Gromadzińska J, Wąsowicz W. Today's oxidative stress markers. Med Pr 2015; 66: 393–405. doi:10.13075/mp.5893.00137

[39] Hybertson BM, Gao B, Bose SK, McCord JM. Oxidative stress in health and disease: The therapeutic potential of *Nrf2* activation. Mol Aspects Med 2011; 32: 234–246. doi:10.1016/j.mam.2011.10.006

[40] Hotta S, Uchiyama S, Ichihara K. Brazilian red propolis extract enhances expression of antioxidant enzyme genes *in vitro* and *in vivo*. Biosci Biotechnol Biochem 2020; 84: 1820–1830. doi:10.1080/09168451.2020.1773756

[41] Haas MJ, Onstead-Haas LM, Szafran-Swietlik A, Kojanian H, Davis T, Armstrong P, Wong NCW, Mooradian AD. Induction of hepatic apolipoprotein A–I gene expression by the isoflavones quercetin and isoquercetrin. Life Sci 2014; 110: 8–14. doi:10.1016/j.lfs.2014.06.014

[42] Mulvihill EE, Allister EM, Sutherland BG, Telford DE, Sawyez CG, Edwards JY, Markle JM, Hegele RA, Huff MW. Naringenin prevents dyslipidemia, apolipoprotein B overproduction, and hyperinsulinemia in LDL receptor-null mice with diet-induced insulin resistance. Diabetes 2009; 58: 2198–2210. doi:10.2337/db09-0634

[43] Kim YW, Byzova TV. Oxidative stress in angiogenesis and vascular disease. Blood 2014; 123: 625–631. doi:10.1182/blood-2013-09-512749

[44] Moldogazieva NT, Mokhosoev IM, Mel'nikova TI, Porozov YB, Terentiev AA. Oxidative stress and advanced lipoxidation and glycation end products (ALEs and AGEs) in aging and age-related diseases. Oxid Med Cell Longev 2019; 2019: 3085756. doi:10.1155/2019/3085756

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[45] Lapchak PA, Zhang JH, Noble-Haeusslein LJ. RIGOR Guidelines: escalating STAIR and STEPS for effective translational research. Transl Stroke Res 2013; 4: 279–285. doi:10.1007/s12975-012-0209-2

[46] Landis SC, Amara SG, Asadullah K, Austin CP, Blumenstein R, Bradley EW, Crystal RG, Darnell RB, Ferrante RJ, Fillit H, Finkelstein R, Fisher M, Gendelman HE, Golub RM, Goudreau JL, Gross RA, Gubitz AK, Hesterlee SE, Howells DW, Huguenard J, Kelner K, Koroshetz W, Krainc D, Lazic SE, Levine MS, MacLeod MR, McCall JM, Iii RTM, Narasimhan K, Noble LJ, Perrin S, Porter JD, Steward O, Unger E, Utz U, Silberberg SD. A call for transparent reporting to optimize the predictive value of preclinical research. Nature 2012; 490: 187–191. doi:10.1038/nature11556

[47] Marques DVB, Felizardo AA, Souza RLM, Pereira AAC, Gonçalves RV, Novaes RD. Could diet composition modulate pathological outcomes in schistosomiasis mansoni? A systematic review of *in vivo* preclinical evidence. Parasitology 2018; 145: 1127–1136. doi:10.1017/S0031182018000057

[48] Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, Shamseer L, Tetzlaff JM, Akl EA, Brennan SE, Chou R, Glanville J, Grimshaw JM, Hróbjartsson A, Lalu MM, Li T, Loder EW, Mayo-Wilson E, McDonald S, McGuinness LA, Stewart LA, Thomas J, Tricco AC, Welch VA, Whiting P, Moher D. The PRISMA 2020 statement: An updated guideline for reporting systematic reviews. BMJ 2021; 372: n71. doi:10.1136/bmj.n71

[49] Bankova V. Chemical diversity of propolis and the problem of standardization. J Ethnopharmacol 2005; 100: 114–117. doi:10.1016/j.jep.2005.05.004

[50] Bankova V, Popova M, Trusheva B. Propolis volatile compounds: chemical diversity and biological activity: a review. Chem Cent J 2014; 8: 28. doi:10.1186/1752-153X-8-28

[51] Eriksen MB, Frandsen TF. The impact of PICO as a search strategy tool on literature search quality: A systematic review. J Med Libr Assoc 2018; 106: 420–431

[52] Hooijmans CR, Rovers MM, Vries RBM, Leenaars M, Ritskes-Hoitinga M, Langendam MW. SYRCLE's risk of bias tool for animal studies. BMC Med Res Methodol 2014; 14: 43. doi:10.1186/1471-2288-14-43

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