



Potential Role of Propolis Flavonoid on Malondialdehyde and Superoxide Dismutase Levels on Endometriosis

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Abstract

Endometriosis is a prevalent gynecological condition that affects around 10% of women within the reproductive age group globally. Recent research indicates that oxidative stress plays a significant role in the development of endometriosis. The present administration of progestin hormone treatment has been shown to induce additional oxidative stress, which is characterized by elevated levels of oxidative stress indicators, including malondialdehyde (MDA), and a reduction in the enzymatic antioxidant superoxide dismutase (SOD). The presence of hormonal imbalances in conjunction with these alterations fosters an environment conducive to the metastasis of endometrial cells. This process initiates inflammatory pathways, angiogenesis, and the formation of lesions and tumors, ultimately exacerbating the state of endometriosis. Research findings indicate that propolis has inherent antioxidant properties, characterized by a high concentration of flavonoid components and phenolic acids. Propolis contains active compounds that have the ability to trap free radicals by forming more stable molecules. Propolis has anti-inflammatory, antimicrobial, and immunomodulatory characteristics, hence potentially enhancing the patient's oxidative stress state, mitigating morbidity, and reducing the duration of hospitalization. This research aims to assess the possible impact of flavonoids found in propolis on the treatment of endometriosis.

Introduction

Endometriosis is a persistent inflammatory condition characterized by the proliferation of aberrant tissue that has resemblance to the endometrium, extending beyond the confines of the uterine cavity, hence inducing inflammation. The predominant symptoms of endometriosis are pain and infertility, however it is not rare for the condition to manifest asymptotically. The reported complaints include a range of symptoms, such as abnormal or excessive bleeding, gastrointestinal disturbances, dysmenorrhea, dyspareunia, dysuria, dyschezia, and pelvic discomfort. According to Puspasari et al. (2007) and Nnoaham et al. (2011), The process of diagnosing endometriosis often takes a significant amount of time, resulting in risk factors for endometriosis mostly reflecting the outcomes of the illness rather than the underlying causes (Ashrafi et al., 2016). Several factors have been identified as potential contributors to the increased risk of endometriosis. These factors include advancing age, alcohol consumption, early onset of menstruation, a family history of endometriosis, infertility, concurrent menstrual intercourse, low body weight, prolonged menstrual flow, and short menstrual cycle intervals.

Conversely, engaging in regular exercise has been found to provide a protective effect against endometriosis. The references used are (Ashrafi et al., 2016; Gupta et al., 2015; Peterson et al., 2013; and Becker et al., 2020). Endometriosis in women leads to alterations in their quality of life due to pelvic discomfort. Endometriosis, being a chronic inflammatory condition, requires ongoing care that focuses on optimizing the use of medicinal therapy while limiting the need for multiple procedures.

This approach aims to minimize problems and morbidity in patients. The references used are Gupta et al. (2015) and Becker et al. (2020). The worldwide occurrence of endometriosis varies between 2% and 11% among women without symptoms, and between 5% and 50% among women with infertility. However, the prevalence rate in Indonesia remains uncertain due to the absence of comprehensive epidemiological investigations conducted on a national level. Between 2000 and 2005, RSUPN dr. Cipto Mangunkusumo Jakarta recorded 111 instances of endometriosis. The average age of the patients was 33 years, and 48.4% of them had infertility. In the period of 2017-2019, the Central General Hospital (RSUP) Dr. M. Djamil Padang recorded a total of 195 individuals diagnosed with endometriosis. Among these patients, 85.7% were within the reproductive age range, while 27.6% reported having infertility (Puspasari et al., 2007; Tifani et al., 2021).

Oxidative Stress in Endometriosis

The maintenance of a balance between oxidants and antioxidants in tissues is crucial in order to prevent the occurrence of severe oxidative stress. Oxidative stress in pathological conditions such as cancer, atherosclerosis, neurological degradation, and chronic inflammation, particularly in endometriosis, is characterized by cytotoxic and mutagenic oxidation processes in lipids, proteins, and deoxyribonucleic acid (DNA). These processes are induced by free radicals and reactive oxygen species (ROS) (Wyatt et al., 2023). Endogenous production of both free radical and non-free radical molecules is seen in reactive oxygen species (ROS). Unpaired electrons in free radicals, including singlet oxygen (1O_2), hydrogen peroxide (H_2O_2), superoxide, hydroxyl radicals, and nitric oxide (NO), contribute to their high reactivity. These molecules have the ability to directly interact with lipids, proteins, and nucleic acids, leading to alterations in their composition. Consequently, this may lead to the instability of lipid membranes, misfolding of proteins, and damage to DNA. According to Gupta et al. (2012) and Clower et al. (2022), recent study indicates that oxidative stress plays a significant role in the development of endometriosis. ROS molecules modify the permeability of endothelial cells and the production of adhesion molecules, therefore initiating inflammatory processes.

The pathogenesis of endometriosis is influenced by oxidative stress, which is facilitated by the activation of macrophages. This activation may exacerbate oxidative stress conditions by generating lipid peroxides and other by-products coming from the interactions between apolipoproteins and peroxides. The confluence of these activities will elevate the concentration of proinflammatory mediators, thereby initiating further inflammation in endometriosis. The references used are (Gupta et al., 2012; Gupta et al., 2006; Vitale et al., 2018). The development of endometriosis may be categorized into three distinct phases: genetic inheritance from parents, epigenetic alterations, and iron excess. It is postulated that the genesis of endometriotic tissue may be attributed to retrograde menstruation, coelomic metaplasia (peritoneal lesions), and lymphatic and vascular metastases (extra pelvic lesions). According to the coelomic metaplasia hypothesis, the ovarian germinal epithelium endeavors to replicate the process of endometrium creation that originates from the Müllerian duct. This process is initiated by endogenous biochemical stimuli, leading to the development of endometrial tissue and undifferentiated cells. The references used are Gupta et al. (2015) and Becker et al. (2020). Halban's 1925 metastasis hypothesis posited that endometrial cells had the ability to disseminate by lymphogenous and hematogenous routes, resulting in the development of endometriotic lesions beyond the peritoneal cavity. Endometriotic lesions manifest as epithelial

glands and stromal cells that have physiological similarities to the normal functioning of endometrial tissue. Endometriosis may be influenced by several hormonal variables, including elevated levels of estrogen and reduced levels of progesterone, exposure to dioxin pollutants, immunological dysregulation, and chronic inflammation. The references used are Gupta et al. (2015) and Becker et al. (2020).

Iron production from lysed red blood cells in endometriotic lesions during menstruation is responsible for the generation of oxidative stress in endometriosis. The buildup of iron resulting from retrograde menstruation may give rise to a range of cytotoxic consequences. This is because it has the potential to disturb the equilibrium between the generation of free radicals and the defense mechanisms against them, so inducing oxidative stress. Consequently, this process contributes to the development of endometriosis. Oxidative stress induced by iron arises from the generation of harmful hydroxyl radicals within an environment characterized by an abundance of iron. This leads to the buildup of somatic mutations via oxidative stress, which is facilitated by the Fenton reaction. Endometrial lesions and peritoneal macrophages may experience iron buildup. Macrophages phagocytose erythrocytes, resulting in the release of hemoglobin (Hb) into the peritoneal fluid.

This Hb may either form a compound with haptoglobin or undergo catabolism by hemeoxygenation-1, leading to the production of free iron. Iron that is not bound may be absorbed for the purpose of storage, or it can be transported by proteins like ferritin and transferrin, resulting in the buildup of iron inside macrophages. Iron, a potent oxidizing agent, may initiate a cascade of oxidative stress, leading to a cascade of variables that exacerbate local inflammation and promote the rapid transformation of ectopic endometrial cells into malignant cells. Iron often regulates many genes implicated in the development and advancement of endometriosis, as well as the body's reaction to oxidative stress. The presence of an excessive amount of iron does not have a direct impact on the development of lesions. However, it does play a role in the progression of endometriosis by promoting cellular proliferation inside the lesion (Gupta et al., 2015). Increased generation of reactive oxygen species (ROS) is linked to higher rates of proliferation in both endometriotic cells and tumor cells.

Different samples are collected to get oxidative stress indicators, which are categorized into five primary groups: serum, peritoneal fluid, follicular fluid, ovarian cortex, and endometrial tissue. There is a potential association between lipid metabolism and inflammatory variables, which may contribute to the onset of oxidative stress. Elevated levels of lipid oxidation end products, also known as lipid peroxidation, are often used as indicators of oxidative stress. Polyunsaturated fatty acids, particularly arachidonic acid (AA), are very vulnerable to oxidative damage when exposed to reactive oxygen species (ROS) or free radicals due to the existence of unsaturated double bonds, according to Harlev et al. (2015) and Scutiero et al. (2017). Research findings indicate that women afflicted with this particular ailment exhibit significantly reduced concentrations of the enzymatic antioxidants superoxide dismutase and glutathione peroxidase inside their peritoneal fluid in comparison to women who are in good health. These two antioxidants play a crucial role in the mechanism of free radical degradation. It is postulated that a diet rich in antioxidants might be beneficial for women suffering from endometriosis. Endometriosis patients exhibiting both superficial and deep infiltration demonstrated elevated levels of oxidative stress markers in their peritoneal fluid when compared to women without the condition.

This was primarily attributed to an increased presence of iron in the peritoneal fluid, which can be attributed to a higher quantity of erythrocytes resulting from retrograde menstruation. It is shown that these conditions induce oxidative stress in the primary location of the ectopic lesion, hence promoting the development of further lesions. In contrast, endometriotic lesions that have already developed exhibit elevated iron levels, accompanied by persistent oxidative stress, leading to further lesion progression and the manifestation of endometriosis symptoms. This

phenomenon is further intensified by a reduction in the efficacy of preventive systems against cellular harm, thereby initiating the progression of endometriosis. The studies conducted by Gupta et al. (2012), Duca et al. (2019), Harlev et al. (2015), Scutiero et al. (2017), and Wyatt et al. (2023) have been referenced.

Therapeutic Approach For Endometriosis And Its Side Effect

The primary emphasis in the treatment of endometriosis is the control of pain. The prevalence of pain among endometriosis patients is reported to be 92%, necessitating ongoing care or recurrent pain medication due to the reoccurrence of symptoms, even after surgical intervention. The selection of therapeutic interventions for individuals with endometriosis might vary based on factors such as reproductive status, medication tolerance, drug accessibility, and the financial implications associated with endometriosis treatment (Gezer & Oral, 2015). Progestin hormone preparations provide a viable therapeutic approach for managing pain associated with endometriosis, and are extensively used in developing nations such as Indonesia. These implants are efficacious in alleviating pain symptoms associated with endometriosis, with the benefit of a lifespan of up to 5 years and requiring little expenses. Nonsteroidal anti-inflammatory medicines (NSAIDs) and a combination of contraceptive tablets are other primary treatment options that may be used either individually or in conjunction with one another. GnRH analogues and aromatase inhibitors are often used as second-line therapeutic options. In cases when pain continues to persist after medication intervention, surgical interventions may be considered, such as lesion excision or ablation, as well as adhesion lysis. The citations are (Gunardi et al., 2021; Supriyadi et al., 2017).

The experience of pain in individuals with endometriosis may be attributed to several sources, including active bleeding originating from endometriotic lesions, an overexpression of growth hormones and proinflammatory cytokines, and injury to the pelvic nerves. The use of progestin hormone in the context of endometriosis has the potential to elicit anovulation and endometrial shrinkage, impede the development of new blood vessels, and activate anti-inflammatory mechanisms. Progestin agonists also lower the frequency of GnRH release, resulting in a reduction in the production of FSH and LH. Due to this impact, prolonged administration of the progestin hormone will inhibit the production of steroids in the ovaries, leading to anovulation and reduced levels of ovarian steroids. Prolonged hypoestrogenic and hypergestagenic circumstances may lead to the conversion of eutopic and ectopic endometrium into decidual structures. The occurrence of excessive bleeding as a side effect might be attributed to the low blood estradiol levels that are a consequence of ongoing progestin medication (Gezer & Oral, 2015; Gunardi et al., 2021). Individuals who utilize progestin hormones and subsequently develop bleeding issues are recognized to exhibit elevated amounts of lipid peroxides, particularly malondialdehyde (MDA).

The established process inside the endometrium involves an elevation in the creation of epoxide, which serves as a mediator for the liberation of oxygen radicals. Subsequently, oxygen radicals attach to the phospholipid membrane of vulnerable polyunsaturated fatty acids by extracting hydrogen. The subsequent step involves the generation of lipid radicals, which, upon interaction with oxygen, yield lipid peroxides. Subsequently, a cascade of lipid peroxidation will commence, which may be very detrimental if not regulated by maintaining an equilibrium between oxidants and antioxidants. Previous research conducted on vascular smooth muscle cell cultures has demonstrated that the administration of progesterone leads to an elevation in oxidative stress. This is achieved by diminishing the expression and activity of the superoxide dismutase (SOD) enzyme through a transcriptional mechanism. Additionally, progesterone acts as an antagonist to the estrogen-induced upregulation of the SOD enzyme. References: Subakir et al. (2000) and Wassmann et al. (2005).

Malondialdehyde As A Marker Of Oxidative Damage

MDA is considered to be one of the most mutagenic indicators in the context of oxidative damage. The chemical known as MDA is the ultimate outcome generated from the breakdown of arachidonic acid and other bigger polyunsaturated fatty acids, facilitated by either enzymatic or non-enzymatic mechanisms. Enzymatic processes, facilitated by lipoxygenase (LOX) and cyclooxygenase (COX), are responsible for the process of lipid peroxidation. These reactions include the oxidation of AA, resulting in the production of prostaglandin, prostacyclin, thromboxane, and leukotrienes. The process of oxidation mediated by free radicals comprises an autocatalytic chain reaction initiated by reactive oxygen species (ROS), particularly hydrogen peroxide ($\text{HO}\cdot$) and rhodium peroxide ($\text{ROO}\cdot$). These ROS catalyze the reduction of hydrogen atoms in unsaturated bonds, resulting in the production of carbon radicals. These carbon radicals then combine with oxygen to generate peroxy radicals. Lipid peroxy radicals serve as chain carrier radicals in the chain reaction, resulting in the production of lipid hydroperoxides. When transition metals are present, lipid hydroperoxides have the ability to generate alkoxy radicals as well as $\text{ROO}\cdot$ and $\text{HO}\cdot$. This mechanism ensures the uninterrupted progression of the chain oxidation reaction, resulting in the production of short chain oxidation products such as diverse aldehydes, alkanes, and alkenes (Ayala et al., 2014; Harlev et al., 2015; Marrocco et al., 2017; Scutiero et al., 2017).

The enzymatic process of synthesizing thromboxane A₂ (TXA₂), and 12-l-hydroxy-5,8,10-acid. heptadectrienoate (HHT), from the decomposition of arachidonic acids and larger polyunsaturated fatty acids (PUFA), produce MDA molecules (blue color path). The production of prostaglandin H₂ (PGH₂) occurs via the enzymatic reaction between the cyclooxygenase enzyme (1) and arachidonic acid. TXA₂ is produced by the enzymatic interaction between thromboxane A₂ synthase (3) and PGH₂. The first step in the progression from arachidonic acid to prostaglandin and thromboxane involves the conversion of Prostaglandin G₂ (PGG₂) into Prostaglandin Glutamic Acid (PGH₂) by the action of prostacyclin hydroperoxidase (2) (Ayala et al., 2014). The production of MDA molecules may also occur via non-enzymatic mechanisms, namely via the brown color pathway, as a result of lipid peroxidation.

The process of lipid peroxidation results in the formation of lipid hydroperoxides, which then undergo a cyclization process with peroxy radicals to generate new oxygen radicals. Oxygen radical molecules have the ability to undergo further cyclization, resulting in the formation of bicyclic endoperoxide. Additionally, they may undergo cleavage to produce MDA. Subsequently, the MDA molecule undergoes metabolic processes including oxidation facilitated by aldehyde dehydrogenase (4), followed by decarboxylation mediated by the decarboxylase enzyme (5), resulting in the formation of acetaldehyde. Acetaldehyde undergoes oxidation by the action of aldehyde dehydrogenase (4), resulting in the formation of acetate. Acetate is then transformed into acetyl CoA by acetyl CoA synthase (6), after which it proceeds through the tricarboxylic acid cycle (7) to yield carbon dioxide (CO₂) and water (H₂O). Ayala et al. (2014) and Sorensen et al. (2013) are the sources cited.

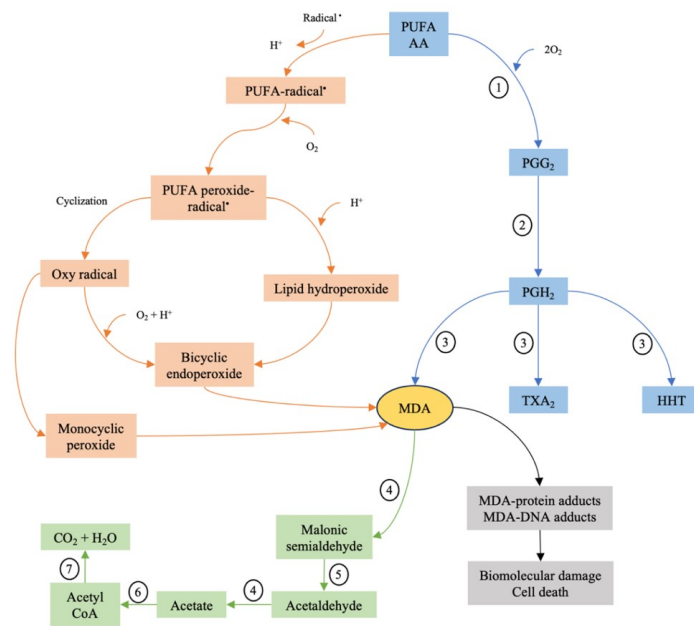


Figure 1. Formation of MDA from Polyunsaturated Fatty Acids

Source: has been reprocessed from reference (Ayala et al., 2014)

Description: PUFA: polyunsaturated fatty acids, AA: arachidonic acid, PGG₂: prostaglandin G₂, PGH₂: prostaglandin H₂, TXA₂: thromboxane A₂, HHT: 12-l-hydroxy-5,8,10heptadecaricenoic acid. Important enzymes in the formation and metabolism of MDA: cyclooxygenase (1), prostacyclin hydroperoxidase (2), thromboxane A₂ synthase (3), aldehyde dehydrogenase (4), decarboxylase (5), acetyl CoA synthase (6), tricarboxylic acid cycle (7).

After its formation, malondialdehyde (MDA) may undergo enzymatic metabolism or interact with biological proteins, tissues, or DNA. The aldehyde groups of MDA exhibit reactivity towards nucleophiles, enabling the formation of adducts that induce biomolecular damage. The capacity of MDA to generate adducts is then used to quantify MDA concentrations. The TBARS test is a widely used technique for the detection of lipid peroxidation. Thiobarbituric acid is capable of detecting the formation of a pink product when malondialdehyde molecules combine with two thiobarbituric acids (MDA-TBA₂). The first step in the chemical examination of MDA involves its quantification as a constituent of TBARS, which is used to evaluate lipid peroxidation by the utilization of a spectrophotometer or fluorometer. When exposed to acidic conditions and high temperatures over long periods of time, a single MDA molecule undergoes a reaction with two TBA molecules, resulting in the formation of the MDA-TBA₂ adduct. This adduct is a highly visible molecule with a maximum wavelength of 532 nm and exhibits fluorescence with an excitation wavelength of 515 nm and an emission wavelength of 553 nm according to Marrocco et al. (2017) and Mas-Bargues et al. (2021).

According to Nasiri et al. (2017), women with endometriosis have elevated levels of serum MDA in comparison to healthy individuals. Supplementation with vitamins C and E, which are natural antioxidants with low levels in women with endometriosis, leads to a reduction in levels of MDA and lipid hydroperoxides (LOOH) in their blood and peritoneal fluid (Mier-Cabrera et al., 2008).

The Role of Superoxide Dismutase as an Endogenous Antioxidant

Oxidative stress may arise due to the dysregulation of several components, including enzymes responsible for the generation of reactive oxygen species (ROS), or alterations in the antioxidant defense system as a consequence of heightened redox stress. These metrics may be used to evaluate the body's redox equilibrium or in the presence of certain illnesses. The

components implicated in the domain of redox proteomics include cysteine residues, namely glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). Additionally, enzymes responsible for generating reactive oxygen species (ROS) and transcription factors that play a role in regulation are also considered (Marrocco et al., 2017).

The defense against ROS production consists of three steps. Enzymatic inactivation is the first step. The evaluation of the first defense mechanism against oxidative stress included the assessment of antioxidant capacity by the measurement of both enzymatic and non-enzymatic antioxidants. The subsequent phase involves the amalgamation of DNA base damage and enzymes that facilitate the hydrolysis of nucleotide oxidation. The third phase involves the restoration of DNA that has undergone oxidative damage. Superoxide Dismutase (SOD) functions as the primary intracellular antioxidant enzyme, serving as the first barrier against the deleterious impacts of accumulated reactive oxygen species (ROS). Consequently, it assumes the crucial role of detoxifying the body. The SOD protein is a metalloenzyme that provides protection against up to 97% of targets that are attacked by superoxide anions. SOD, an essential component of the enzyme defense system, is found in almost all cells throughout the body. Mitochondria serve as the primary origin of reactive oxygen species (ROS) across all cellular types. Superoxide ($O_2^{\bullet-}$) is primarily produced inside the mitochondrial electron transport chain. It may be transformed into hydrogen peroxide (H_2O_2) and molecular oxygen (O) by the action of superoxide dismutase (SOD), or it can undergo spontaneous dismutation ($2 HO_2 \rightarrow O_2 + H_2O_2$).

The superoxide dismutase (SOD) enzyme functions by facilitating the reduction and oxidation of superoxide, a process known as disproportionation. This mechanism is crucial in suppressing the development of peroxynitrite, as well as preventing endothelial and mitochondrial dysfunction. Carmo de Carvalho e Martins et al. (2022) conducted a study. Superoxide dismutase (SOD) enzymes encompass a collection of enzymes comprising three distinct isoforms, each characterized by varying cellular localization and metal cofactors. These isoforms include Cu/Zn-SOD homodimer (SOD-1), which is found in the cytosol and mitochondrial intermembrane space; homotetrameric Cu/Zn-SOD (SOD-3), which is distributed outside the cell; and homotetrameric Mn-SOD (SOD-2), which is localized within the mitochondria. The measurement of superoxide dismutase (SOD) activity involves the analysis of the reduction rate of tetrazolium salt by oxygen ions ($O_2^{\bullet-}$) generated by the xanthine oxidase enzyme system. Carvalho et al. (2012), Marrocco et al. (2017), and Scutiero et al. (2017) have been cited in the text. The activity of the SOD enzyme in plasma may be quantified as U/L. The definition of one unit of superoxide dismutase (SOD) activity is the quantity of enzyme necessary to dismutate 50% of the superoxide radicals that are present. The activity of superoxide dismutase (SOD) may also be measured in erythrocyte lysate and translated into grams of hemoglobin, which is then represented as U/g Hb, according to Ściskalska et al. (2020).

Individuals with endometriosis have reduced activity of superoxide dismutase (SOD) in their plasma, suggesting a decline in their antioxidant ability. The oxidative stress in the follicular fluid of women diagnosed with endometriosis was assessed by Nasiri et al. (2017). The follicular fluid of women with endometriosis exhibited elevated levels of ROS, MDA, and NO. Immature oocytes and low quality embryos are linked to elevated levels of reactive oxygen species (ROS) and nitric oxide (NO). Women with endometriosis have reduced activity in their antioxidant system. The study revealed a decrease in enzymatic antioxidant capabilities, including superoxide dismutase (SOD), catalase, glutathione peroxidase, and glutathione reductase, among women diagnosed with endometriosis. According to Amreen et al. (2019), Marrocco et al. (2017), and Scutiero et al. (2017).

The pathophysiology of endometriosis is significantly influenced by oxidative stress, prompting several studies to prioritize the reduction of oxidative stress as a therapy objective

for this condition. Propolis, sometimes referred to as 'bee glue', is a substance derived by bees that has functional attributes, such as its use as a chemical weapon by honey bees. This substance has been recognized and employed by human beings since around 300 BC. Propolis, derived from the Greek terms pro and polis, refers to a chemical used by bees to protect their hives. Pro means 'in front of' or 'at the entrance to', while polis denotes 'community' or 'city'. The direct consumption of propolis as a food source is not feasible due to its resinous nature, characterized by a brownish hue.

This material is obtained by worker bees via the collection of plant components, including leaf pieces and bark from various tree species such as birch, poplar, pine, alder, willow, and palm trees, which serve as habitats or visitation sites for the bees. In their hives, bees gather resin exudates from plants, which undergo modification via the action of bee enzymes. This modification involves the addition of wax and glucosidation enzymes present in bee saliva, resulting in the formation of propolis. Subsequently, the propolis will serve as a building agent for beehives. Bankova et al. (2000), Kocot et al. (2018), Mello et al. (2010), and Pasupuleti et al. (2017) have been cited in the text. Propolis is well recognized as a natural reservoir of antioxidants that possess the capability to counteract reactive oxygen species (ROS) and mitigate the detrimental impacts of oxidative stress, which serves as a fundamental factor in the development of several illnesses. Propolis contains phenolic chemicals that contribute to the removal of free radicals under oxidative stress. Numerous scholarly investigations have extensively emphasized the antioxidant characteristics of propolis and its potential use in the treatment of diverse ailments, such as endometriosis (Bankova et al., 2000; Braakhuis, 2019).

Polyphenol Compounds and Active Substances Contained in Propolis

Propolis has a significant function in mitigating the transmission of microbial diseases, including both bacteria and fungus, as well as defending against predatory assaults. The Egyptians used the anti-decay characteristics of propolis to safeguard their bodies from decomposition. Similarly, the Greeks and Romans employed propolis as an antiseptic and traditional medicine for wound treatment. Since the 17th century, propolis has been recognized as an official medicinal substance in the London pharmacopoeia. It is often used in the health business, either in its pure form or in conjunction with other natural constituents (Bhargava et al., 2021). Propolis comprises almost 300 known chemicals. Phenolic acids, flavonoids, terpenes, waxes, vitamins, proteins, amino acids, and carbohydrates are among the several components that make up the big group.

The majority of propolis constituents consist of phenolic compounds, irrespective of the propolis's kind and source. Phenolic component content is often used as a measure for assessing the quality of propolis. Phenolic compounds include a variety of chemical constituents, including phenolic acids, phenolic aldehydes, phenols and esters, ketophenols, coumarins, as well as additional constituents such as eugenol, anethole, hydroquinone, pterostilbene, and naphthalene. Flavonoids are a significant category within propolis. Flavonoids represent a distinct category of phytochemicals that are abundantly present in a wide range of plant species and plant-derived commodities, including vegetables, fruits, tea, and spices. The predominant structure of flavonoids consists of glycosides, whereby phenolic groups are connected to one or more sugar groups via glycosidic linkages. There are around 13 categories of flavonoids and over 4000 distinct types of chemicals. Among the several classes of phytonutrients, there are six classes and around 20-30 flavonoid compounds that are often present in food. These subclasses are extensively ingested and provide advantageous biological effects due to their antioxidant capabilities (Sefrina et al., 2020). Flavonoids are present in plants in the form of aglycones derived from plant glycoside compounds. Bees release β -glucosidase when they gather propolis, which breaks down the glycosides of flavonoids into aglycones and sugars. Propolis contains various flavonoids such as tectochrysin, pinocembrin, chrysin, quercetin, galangin, genkwanin, apigenin, kaempferol, and 5-hydroxy-4',7-dimethoxyflavone.

Additionally, it contains caffeic acid phenethyl ester (CAPE), which acts as an inhibitor of the pro-inflammatory nuclear factor-kappa b (NF- κ B) pathway, thereby reducing inflammation and oxidative stress. Bankova et al. (1983), Bhargava et al. (2021), Braakhuis (2019), and Kurek-Górecka et al. (2014) are the references cited in the text.

Flavonoids exhibit distinct subgroupings based on the positioning of the carbon attachment on the C ring to which the B ring is connected, as well as the extent of unsaturation and oxidation of the C ring. Isoflavones are flavonoids in which the B ring is connected to position 3 of the C ring. Neoflavonoids are compounds in which the B ring is attached to position 4, while compounds with the B ring attached to position 2 may be classified into many subgroups depending on the structural properties of the C ring. The subgroups identified in the study conducted by Panche et al. (2016) include flavones, flavonols, flavanones, flavanonols, flavanols or catechins, anthocyanin, and chalcone. Propolis is composed of several substances, such as volatile oils and aromatic acids (5-10%), wax (30-40%), resin, balsam, and pollen grains. These constituents serve as significant sources of magnesium, nickel, calcium, iron, and zinc. Propolis is additionally saturated with biometabolites such as amino acids, sugars, nucleic acids, and lipids. It also contains minerals including magnesium, calcium, iron, potassium, sodium, copper, zinc, manganese, and iron.

Additionally, propolis contains hydrocarbon compounds such as alkanes, alkenes, alkadienes, monoesters, diesters, and aromatic esters. Furthermore, propolis contains vitamins B1, B2, B6, C, and E, which play crucial roles in the structures and functions of various living systems. Various varieties of propolis have been shown to include enzymes such as succinate dehydrogenase, glucose-6-phosphate, adenosine triphosphate, and phosphoric acid (Bhargava et al., 2021; Braakhuis, 2019; Cauich-Kumul & Campos, 2019). The use and standardization of propolis in the health business provide a difficulty due to the significant heterogeneity seen in the chemical makeup of different types derived from diverse geographical and botanical locations. The references used are Bhargava et al. (2021), Braakhuis (2019), and Cauich-Kumul & Campos (2019). Polyphenols have antioxidant action via many routes. The first mechanism involves the inhibition of oxidative enzyme activity, resulting in the suppression of reactive oxygen species (ROS) formation. The subsequent step involves the interaction between metal ions, which play a crucial role in the generation of free radicals. As scavengers of reactive oxygen species (ROS) such as OH \cdot , hydrogen peroxide (H₂O₂), superoxide anions (O₂ \cdot^-), perhydroxyl radicals (HO₂ \cdot), and ROO \cdot , polyphenols have the ability to disrupt the oxidative stress reaction cascade that ultimately leads to lipid peroxidation. Furthermore, polyphenols are known to contribute to the enhancement of intracellular gap junction communication, including their synergistic effects when combined with other antioxidants. The studies conducted by Carmo de Carvalho e Martins et al. (2022), Kurek-Górecka et al. (2014), and Oršolić & Jazvinščak Jembrek (2022) have been referenced.

Polyphenol compounds has the capacity to impede the functionality of enzymes involved in the generation of reactive oxygen species (ROS) via the reduction of xanthine oxidase activity. Xanthine oxidase is an oxidase that catalyzes the reduction of NADPH, hence facilitating the production of superoxide anion radicals. Ascorbic acid oxidase, COX-1 and COX-2, 5-LOX, 12-LOX, 15-LOX, Na⁺/K⁺ ATPase, and cyclic adenosine monophosphate (cAMP) phosphodiesterase are among the enzymes that are inhibited by polyphenolic substances according to Kurek-Górecka et al. (2014). Flavonoids have been observed to enhance the activation of Nrf2, a transcription factor that governs the expression of around 250 genes associated with cellular homeostasis. Nrf2 is responsible for regulating various enzymes, including antioxidant proteins like superoxide dismutase 1 (SOD-1) that play a crucial role in preventing degenerative and inflammatory processes. Nrf2 activation not only triggers antioxidant reactions but also contributes to inflammation, cellular defense, and the maintenance of homeostasis, according to Carmo de Carvalho e Martins et al. (2022).

Currently, there is a lack of clinical trial studies that evaluate the impact of propolis on endometriosis in people. Nevertheless, much research has been conducted on the antioxidant activity of propolis in relation to several chronic diseases. According to a study conducted by Sungkar et al. (2021), the administration of ethanol extract of propolis (EEP) to mice at a dosage ranging from 200 to 800 mg per kgBW per day for a duration of seven days resulted in a decrease in oxidative stress biomarkers, including blood MDA and interleukin-6 (IL-6), while concurrently increasing levels of vascular endothelial growth factor (VEGF). Ridwan et al. (2015) demonstrated that the abundant flavonoid content in propolis has the ability to decrease reactive oxygen species (ROS) generated as a result of hyperglycemic circumstances in diabetes. Administering propolis at a dosage of 50-175 mg/kgBW may decrease the density of reactive oxygen species (ROS) and enhance the generation of insulin in mice with diabetes. The study conducted by Sahlan et al. (2021) demonstrated that propolis, when administered at doses of 100 mg/kg and 200 mg/kg, contains flavonoid compounds that exhibit hepatoprotective properties.

These effects are thought to mitigate hepatocyte damage resulting from lipid peroxidation, as flavonoids function as antioxidants, leading to reduced ALT (alanine transaminase) levels. In a study done by Jasprica et al. (2007), a total of 47 healthy women and men were tested to examine the impact of consuming powdered propolis extract for a duration of 30 days on their antioxidant status. The research demonstrated that propolis had positive effects on the male population, resulting in a 23.2% reduction in MDA levels and a 20.9% enhancement in SOD activity after propolis administration. Soleimani et al. (2021) conducted a study on the effects of vigorous physical activities on oxidative stress and inflammation in healthy male participants. They administered a single dosage of 450 mg propolis twice day for a duration of four weeks to healthy and physically active individuals. In comparison to the placebo group, the propolis group exhibited significant alterations in plasma concentrations of IL-6, total oxidant status, total antioxidant capacity, MDA, oxidative stress index, and glutathione, according to Soleimani et al. (2021).

The research conducted by Kanazashi et al. (2023) shown that the administration of propolis supplements has the potential to decrease the buildup of body fat, elevate adipokine levels in the bloodstream, and diminish reactive oxygen species (ROS) levels in old females. A study done by Afsharpour et al. (2019) was a double-blind, randomized clinical trial including individuals diagnosed with type 2 diabetes mellitus. The participants were administered either oral propolis or a placebo at a total daily dosage of 1500 mg for a duration of eight weeks. A significant augmentation in superoxide dismutase (SOD) activity and overall antioxidant capacity was seen in the findings. An additional investigation employing oral propolis supplementation yielded noteworthy findings, including a notable decrease in carbonyl levels and lactate dehydrogenase activity, alongside an elevation in serum GSH and total polyphenol levels after a duration of 18 weeks, in comparison to the control group. These findings indicate an enhancement in antioxidant function among individuals diagnosed with type 2 diabetes mellitus (Zhao et al., 2016).

The anti-inflammatory and antioxidant properties of propolis were demonstrated in a study conducted by Darvishi et al. (2020) in breast cancer patients undergoing chemotherapy. These patients had previously exhibited elevated levels of pro-inflammatory cytokines, namely tumor necrosis factor-alpha (TNF- α), interleukin-2 (IL-2), and protein carbonyl. Patients who ingested propolis did not exhibit substantial elevations in these indicators in comparison to the control group, indicating a superior equilibrium between oxidants and antioxidants in the propolis-consuming group.

Effect of Propolis Polyphenol Compounds on Endometriosis

The in-silico research conducted by Situmorang et al. (2021) demonstrated that the active constituents of propolis derived from Sulawesi possess strong affinity for receptors implicated in the development of endometriosis, including TNF- α , NF- κ B, estrogen α , estrogen β , progesterone A, progesterone B, and prostaglandin E2. Consequently, these components exhibit favorable anti-inflammatory and apoptotic effects against endometriosis models. Sangenon C, a derivative of flavonoids, is an active molecule with several functions. It is recognized for its anticancer, anti-inflammatory, antibacterial, antiviral, antithrombotic, and immunomodulatory properties.

Additionally, it has been reported that in the context of cancer, sangenon C has the ability to trigger apoptosis in cancer cells, specifically in colon cancer. This is achieved by enhancing the generation of reactive oxygen species and diminishing the production of nitric oxide. These effects are linked to the inhibition of nitric oxide synthase expression and the activation of the mitochondrial apoptosis pathway. Phytoestrogens, such as isoflavones and prenylflavonoids, are a group of foods that contain high levels of flavonoids. These compounds have the ability to interact with estrogen receptors (ER) or influence the function of estrogen in living organisms. Phytoestrogens, such as genistein, possess a non-steroidal chemical composition. However, their ability to bind to estrogen receptors is attributed to the presence of a phenolic ring, particularly the 4'-hydroxyl group. Phytoestrogens have the capacity to function as estrogen antagonists at certain doses, contingent upon the quantity and accessibility of receptors.

This mechanism involves competitive interaction with estrogen receptors, hence impeding the binding process with natural estrogen. In the end, this will decrease the likelihood of developing estrogen-sensitive cancers, according to Janabi et al. (2020) and Youseflu et al. (2020). Okamoto et al. (2015) asserted that the oral administration of propolis has the capacity to elicit estrogenic activity in organs possessing estrogen receptors inside a living organism. Furthermore, the study demonstrated that propolis serves as a valuable dietary source of phytoestrogens and has promise as a possible therapeutic intervention for postmenopausal symptoms. Chrysin, a flavonoid compound present in propolis, has the ability to decrease the growth of endometriotic cells and trigger planned cell death in human endometriotic cells in a laboratory setting. This is achieved by stimulating the stress response in the endoplasmic reticulum, elevating calcium levels in the cytosol of endometriotic cells, and inhibiting signaling pathways. The phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt) pathway has been implicated in the proliferation of endometriotic cells. The citations are Bahat et al. (2022) and Ryu et al. (2019).

Caffeic acid, a phenolic molecule often present in propolis, has been seen to exhibit certain effects on ectopic endometrial cells. These effects include a reduction in endometriotic cell survival, a decrease in MDA levels, and an increase in GSH levels and antioxidant enzyme activity (Jamali et al., 2019).

Dosage, Toxicity, and Side Effects of Propolis

Propolis has long been used in a number of countries and shows safety in its use, although not many studies have been reported with limitations such as lack of standardization of propolis products. Propolis has a low level of acute oral toxicity with a reported LD50 (lethal dose, 50%) ranging from 2000 to 7300 mg/kg in mice. Flavonoids, as one of the main constituents of propolis, are reported to have an oral LD50 in mice of 8000 to 40,000 mg/kg. The No Observed Effect Level (NOEL) value for propolis in mice was found to be 1400 mg/kg/day. If applied to human safety, a safety factor of 1000 was used to account for the lack of chronic toxicity studies. Therefore, a safe dose in humans is 1.4 mg/kg body weight/day or 70 mg/day (in humans with a body weight of 50 kg) (Berretta et al., 2017; Burdock, 1998).

In another study in Brazil on Wistar rat test animals, the LD50 was determined to be 3000 mg/kg after administration for 24 hours, doses below this value did not show signs of toxicity in animals. After administration for 30 days, no differences were found in food and water intake, body weight and animal diuresis. After conversion according to Food and Drug Administration (FDA) guidelines, a safe dose was found to be 1700 mg or 1.7 g/day of propolis for adults (Berretta et al., 2017; Jasprica et al., 2007). In human subjects, various clinical studies have concluded that propolis doses varying from 260.0 mg to 2.87 grams per day in humans can be said to be safe. Propolis toxicity tests show the safety of propolis when consumed repeatedly, and long-term use does not cause damage to blood cells, liver or kidney function (Azis et al., 2018; Bako et al., 2020; Berretta et al., 2017).

Conclusion

Endometriosis is a gynecological disorder experienced by 10% or around 190 million women of reproductive age throughout the world (Becker et al., 2020). Endometrial tissue with a functional layer similar to normal endometrium grows outside the uterine cavity, causing the main symptoms range from pain to infertility in endometriosis, giving significant negative impact on quality of life and the resulting dysfunction. Oxidative stress is known to have a major role in the pathogenesis of endometriosis through several processes which trigger further inflammation in endometriosis. Hormone therapy in endometriosis are known to increase apoptosis of endometriosis lesions, but also increases metabolism, so that prooxidants in the body increase and trigger further oxidative stress, characterized by an increase in oxidative stress markers, such as malondialdehyde (MDA), and a decrease in the enzymatic antioxidant superoxide dismutase (SOD) (Gupta et al., 2006; Harlev et al., 2015).

Lipids in biological membranes and lipoproteins are the main peroxidation targets. MDA as a product of lipid peroxidation, is often used in clinical situations as a marker of oxidative stress, because of its high reactivity and most mutagenic properties (Ayala et al., 2014). Superoxide is the start of a chain reaction in producing other secondary prooxidants. Superoxide dismutase acts as the main enzyme that catalyzes the first step of the detoxification pathway (Sharifi-Rad et al., 2020) Increased oxidative stress can increase the side effects of progestin hormone therapy in the form of abnormal bleeding. Increased oxidative stress accompanied by hormonal imbalance creates an environment for endometrial cells to metastasize, triggering inflammatory pathways, angiogenesis, forming lesions and tumors, thereby aggravating the condition of endometriosis (Gupta et al., 2015; Becker et al., 2020; Hickey et al., 2006; Rocca et al., 2021). Adding foods rich in antioxidants is one way to reduce oxidative stress. Various studies show that propolis is a source of natural antioxidants that are rich in flavonoid compounds and phenolic acids. The active substances contained in propolis are known to be able to capture free radicals through the formation of more stable molecules. Propolis also has antiinflammatory, anti-microbial and immunomodulatory properties, which can improve the patient's oxidative stress status, reduce morbidity and length of hospital stay (Braakhuis, 2019; Kocot et al., 2018).

Based on the results and discussion in this article, endometriosis patients need to be given education regarding a balanced nutritional diet with food ingredients rich in antioxidants that are easy to find and affordable. There is consistent evidence on the potential role of flavonoids from propolis which can meet antioxidant needs practically compared to consuming fruit and vegetables in a large amount. Further study on human population, especially on endometriosis patients is needed to confirm these findings before recommending antioxidant supplementation such as flavonoid rich propolis, to help reduce the inflammatory process and pain complaints in patients.

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