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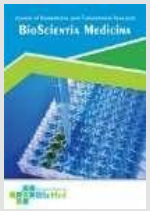

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Propolis Extract Attenuates NF-κB Activation and Chronic Kidney Disease Progression in a Rat Model: Potential Surgical Adjuvant?

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A B S T R A C T

Background: Chronic kidney disease (CKD) is a global health concern often complicated by cardiovascular diseases like atherosclerosis. Both conditions share an inflammatory pathogenesis, with nuclear factor kappa B (NF-κB) playing a central role. Propolis, a natural bee product with anti-inflammatory properties, has shown potential in mitigating CKD progression. This study aimed to investigate the effects of propolis extract on NF-κB activation in a rat model of CKD, exploring its potential benefits as a surgical adjuvant. **Methods:** Male white rats (Rattus norvegicus) were divided into three groups: a control group, a CKD group, and a CKD+Propolis group receiving propolis extract (200 mg/kg body weight) daily for 20 days. CKD was induced using the unilateral ureteral obstruction (UUO) method. NF-κB levels were measured weekly using ELISA. **Results:** Propolis extract significantly reduced NF-κB levels in the CKD+Propolis group compared to the CKD group ($p<0.05$). This effect was consistently observed across all time points, indicating a sustained reduction in NF-κB activation with propolis treatment. **Conclusion:** Propolis extract effectively attenuates NF-κB activation in a rat model of CKD, suggesting its potential as an adjunctive therapy for CKD management, particularly in the context of surgical interventions. Further research is needed to elucidate the underlying mechanisms and evaluate its efficacy in human subjects.

1. Introduction

Chronic kidney disease (CKD) is a significant global health concern characterized by the gradual loss of kidney function over time. The global prevalence of CKD is estimated to be around 10%, with a rising trend due to aging populations and the increasing prevalence of comorbidities such as diabetes and hypertension. CKD not only affects the kidneys but also has systemic consequences, particularly on the cardiovascular system. Cardiovascular disease (CVD) is the leading cause of death in CKD patients, with atherosclerosis playing a major role in this increased risk. Atherosclerosis is a chronic inflammatory disease characterized by the buildup of plaque within the arterial walls, leading to narrowing and hardening of the arteries. This process can result in various cardiovascular complications, including coronary artery disease, stroke, and peripheral artery disease. The pathogenesis of atherosclerosis involves a complex interplay of factors, including lipid accumulation, endothelial dysfunction, and inflammation.[1,2]

Inflammation is a key driver of both CKD and atherosclerosis. In CKD, various factors, such as oxidative stress, uremic toxins, and pro-inflammatory cytokines, contribute to chronic inflammation. This

chronic inflammatory state accelerates the progression of kidney damage and promotes the development of atherosclerosis. Nuclear factor kappa B (NF-κB) is a family of transcription factors that play a central role in regulating inflammatory responses. NF-κB is normally present in the cytoplasm in an inactive form, bound to inhibitory proteins called IκBs. Upon activation by various stimuli, such as pro-inflammatory cytokines, IκBs are phosphorylated and degraded, allowing NF-κB to translocate to the nucleus and activate the transcription of various genes involved in inflammation, immunity, and cell survival.[3-5]

In CKD, NF-κB activation contributes to the progression of kidney damage by promoting the expression of pro-inflammatory cytokines, chemokines, and adhesion molecules. These molecules recruit and activate immune cells, leading to further inflammation and tissue injury. In atherosclerosis, NF-κB activation promotes plaque formation and instability by increasing the expression of adhesion molecules, pro-inflammatory cytokines, and matrix metalloproteinases. Propolis, a natural resinous substance collected by bees from various plant sources, has been recognized for its diverse biological activities, including anti-inflammatory, antioxidant, and antimicrobial properties. Propolis contains a complex mixture of compounds, including flavonoids, phenolic acids, and terpenoids, which contribute to its therapeutic effects.[6-8]

The anti-inflammatory effects of propolis have been attributed to its ability to modulate NF-κB signaling. Propolis can inhibit NF-κB activation through various mechanisms, such as suppressing the degradation of IκBα, preventing NF-κB translocation to the nucleus, and interfering with NF-κB binding to DNA. In vitro and in vivo studies have demonstrated the potential benefits of propolis in attenuating kidney injury and improving renal function. Propolis has been shown to reduce inflammation, oxidative stress, and fibrosis in various experimental models of CKD.[9,10] This study aimed to investigate the effects of propolis extract on NF-κB activation in a rat model of CKD.

2. Methods

This study adhered to the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines to ensure rigorous reporting of experimental design and methodology. All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of Universitas Sebelas Maret and conducted in accordance with ethical guidelines for animal research. This study employed a post-test only control group design, a robust approach in experimental research to assess the effect of a specific intervention by comparing a treated group with a control group after the intervention has been administered. This design minimizes the influence of pre-treatment measurements on the outcome, reducing potential bias and enhancing the internal validity of the study. The study was conducted in two phases; Induction of Chronic Kidney Disease (CKD): This phase was conducted at the Food and Nutrition Study Center Laboratory, Universitas Gadjah Mada, renowned for its expertise in animal research and nutritional studies. This controlled environment ensured the standardization of procedures and minimized external influences on the experimental animals; Assessment of NF-κB Levels: This phase was conducted at the Anatomical Pathology Laboratory, Faculty of Medicine, Universitas Sebelas Maret / Dr. Moewardi Hospital, a leading institution with advanced facilities for immunohistochemical analysis. This collaboration ensured the accurate and reliable measurement of NF-κB levels. The study took place from August to September 2022, providing a defined timeframe for the experiment and minimizing potential seasonal variations that could influence the results.

Male white rats (Rattus norvegicus) were chosen as the experimental animals due to their well-established use in CKD research. Their physiological similarities to humans in terms of renal function and inflammatory responses make them a suitable model for studying CKD progression and potential therapeutic interventions. Specific inclusion criteria were applied to ensure the homogeneity of the study

population; Age: 3-4 months, a period representing young adulthood in rats, minimizing the influence of age-related physiological changes on the results; Weight: 150-300 grams, a healthy weight range for adult male rats, ensuring adequate nutritional status and minimizing potential confounding effects of malnutrition on CKD progression; Source: Rats were obtained from the Faculty of Veterinary Medicine, Universitas Gadjah Mada, a reputable source ensuring the quality and health of the animals. Upon arrival, the rats were acclimatized for one week to the laboratory environment, allowing them to adjust to the new surroundings and minimize stress-induced physiological changes that could influence the results. During this period, they were housed in standard cages under controlled environmental conditions; Light/dark cycle: 12-hour light/dark cycle, mimicking natural light patterns and maintaining normal circadian rhythms; Temperature: 22 ± 2°C, a comfortable temperature range for rats, minimizing thermal stress; Humidity: 55 ± 5% humidity, a suitable range for maintaining respiratory health and preventing dehydration. Throughout the study, the rats were fed a standard BR I diet, a commercially available rat chow that provides balanced nutrition and meets the specific dietary requirements of laboratory rats. They were also provided with ad libitum access to clean drinking water, ensuring adequate hydration and minimizing potential confounding effects of dehydration on renal function.

The minimum sample size required for this study was calculated using a formula that considers the desired level of statistical power, the expected effect size, and the variability within the data. Assuming standard deviation values based on previous research and aiming for a power of 80% and an alpha level of 0.05, the calculated minimum sample size was 7 rats per group. To account for potential attrition or unexpected loss of animals, a total of 24 rats were included in the study, with 8 rats in each of the three groups. The 24 rats were randomly assigned to three groups, ensuring an equal distribution of animals with similar characteristics across the groups and minimizing potential selection bias; Control Group (N): This group consisted of 8 healthy rats that did not undergo any intervention. They served as the baseline for comparison, representing normal physiological function and NF-κB levels in the absence of CKD; CKD Group (K): This group consisted of 8 rats in which CKD was induced using the unilateral ureteral obstruction (UUO) method. They served as the disease control group, representing the progression of CKD and the associated increase in NF-κB levels without any intervention; CKD+Propolis Group (P): This group consisted of 8 rats in which CKD was induced using the UUO method, and they were subsequently treated with propolis extract. This group served to assess the therapeutic potential of propolis extract in attenuating NF-κB activation and CKD progression.

CKD was induced in the CKD and CKD+Propolis groups using the UUO method, a well-established and widely used animal model of CKD. UUO involves the surgical ligation of one ureter, causing obstruction of urine flow and leading to hydronephrosis, tubular atrophy, and interstitial fibrosis, mimicking the pathophysiological changes observed in human CKD. The UUO procedure was performed under aseptic conditions to prevent infection. Rats were anesthetized using a combination of ketamine and xylazine, ensuring adequate anesthesia and minimizing pain during the surgical procedure. A midline abdominal incision was made, and the left ureter was carefully identified and isolated. The ureter was then ligated using a 4-0 silk suture, a non-absorbable suture material that provides secure and permanent obstruction. The abdominal incision was closed in layers using absorbable sutures, minimizing the risk of infection and promoting wound healing.

Propolis extract was prepared using a standardized protocol to ensure consistency and accurate dosing. Commercially available propolis powder was obtained from a reputable supplier, ensuring the quality and purity of the extract. The powder was dissolved in distilled water to achieve a concentration of 200 mg/kg body weight, a dose based on previous research demonstrating therapeutic efficacy in CKD models.

The CKD+Propolis group received propolis extract daily for 20 days, starting from the day of UUO surgery. The extract was administered via oral gavage, a common and effective method for delivering liquid formulations to laboratory animals. This route of administration ensures accurate dosing and minimizes stress to the animals compared to other methods such as intravenous injections. The control and CKD groups received an equivalent volume of distilled water via oral gavage to control for any potential effects of the administration procedure itself.

NF-κB levels were measured weekly in all three groups to monitor the progression of inflammation and assess the therapeutic effect of propolis extract over time. Blood samples were collected via tail vein puncture, a minimally invasive technique that minimizes discomfort to the animals. Serum was separated from the blood samples and stored at -80°C until analysis, ensuring the stability and integrity of the samples. NF-κB levels were measured using a commercially available ELISA kit, a sensitive and specific method for quantifying protein levels in biological samples. The ELISA kit was chosen based on its established reliability and validity in measuring NF-κB levels in rat serum. The assay was performed according to the manufacturer's instructions, ensuring accurate and reproducible results.

Data were analyzed using SPSS for Windows Version 22, a comprehensive statistical software package widely used in research. Descriptive statistics were used to summarize the data, including mean, standard deviation, minimum, and maximum values. These descriptive measures provide a clear overview of the distribution of NF-κB levels in each group at each time point. One-way ANOVA, a statistical test used to compare means between three or more groups, was used to assess the differences in NF-κB levels among the three groups at each time point. This test allows for the determination of whether there are any statistically significant differences in NF-κB levels among the control, CKD, and CKD+Propolis groups. Tukey's post-hoc test, a multiple comparison test, was used to identify specific group differences if a significant result was found in the ANOVA. This test allows for pairwise comparisons between the groups, determining which specific groups differ significantly from each other in terms of NF-κB levels. The level of significance was set at $p<0.05$, a commonly used threshold in research. This means that a p-value less than 0.05 is considered statistically significant, indicating that the observed differences in NF-κB levels are unlikely to be due to chance alone.

3. Results

Figure 1 illustrates the effect of propolis extract on NF-kB levels in chronic kidney disease (CKD) rats. The CKD group shows significantly elevated NF-kB levels compared to the control group ($p<0.05$). This confirms that CKD induces a significant inflammatory response, as evidenced by the increased activation of NF-kB, a key regulator of inflammation. The CKD+Propolis group, which received propolis extract, exhibits significantly lower NF-kB levels compared to the CKD group ($p<0.05$). This indicates that propolis extract effectively attenuates the inflammatory response in CKD by suppressing NF-kB activation. While the CKD+Propolis group still has slightly higher NF-kB levels than the control group, the difference is not statistically significant. This suggests that propolis extract may help restore NF-kB levels closer to the normal range, further supporting its potential as an anti-inflammatory agent in CKD.

Figure 2 displays the time course of NF-κB levels over four weeks in control rats, rats with chronic kidney disease (CKD), and CKD rats treated with propolis extract (CKD+Propolis). NF-κB levels remain consistently low throughout the four weeks, indicating a stable baseline with minimal inflammation. This demonstrates that the experimental procedures themselves did not induce significant inflammatory responses. NF-κB levels are markedly elevated compared to the control group at every time point. This highlights the persistent inflammatory state associated with CKD, reinforcing that the UUO model successfully induced a chronic inflammatory response. Notably, NF-κB levels appear to increase

progressively over the four weeks, suggesting ongoing and potentially worsening inflammation in the absence of intervention. While NF-κB levels are initially elevated in this group (similar to the CKD group), they show a clear downward trend over time. This suggests that propolis extract effectively counteracts the CKD-induced inflammatory response, leading to a gradual reduction in NF-κB activation. By week 4, NF-κB levels in the CKD+Propolis group appear closer to those of the control group, indicating a potential for propolis to help restore a more normal inflammatory balance.

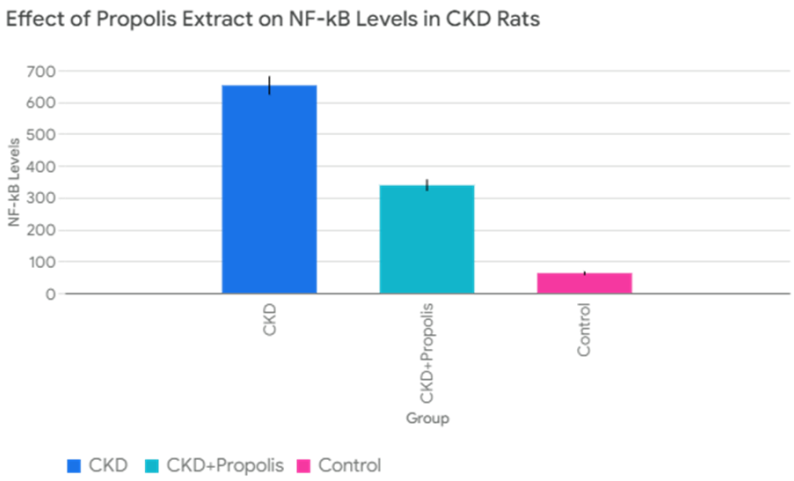

Figure 1. Effect of propolis extract on NF-kB levels in CKD Rats. *p<0.05 CKD VS CKD+Propolis; **p<0.05 CKD VS Control.

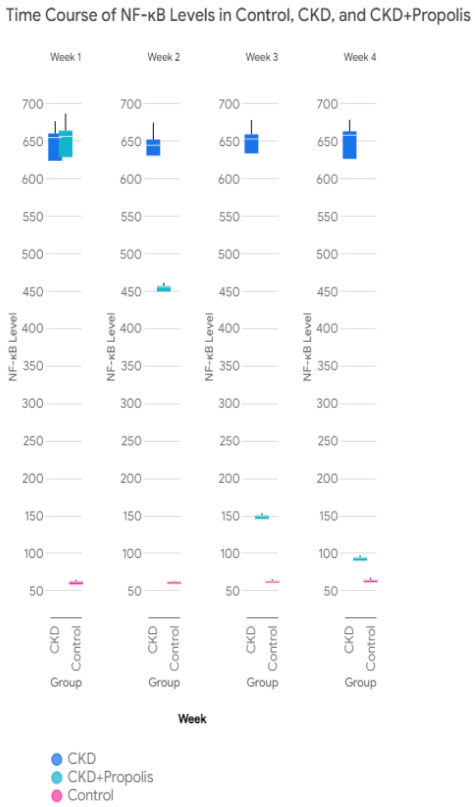

Figure 2. Time course of NF-kB levels.

4. Discussion

Chronic kidney disease (CKD) is a progressive and debilitating condition characterized by the gradual loss of kidney function over time. This insidious disease affects millions globally, imposing a significant burden on individuals, healthcare systems, and societies worldwide. CKD is not merely a disease of the kidneys, it is a systemic disorder with far-reaching implications, particularly for the cardiovascular system. Cardiovascular disease (CVD) is the leading cause of death among CKD patients, and atherosclerosis, a chronic inflammatory condition of the arteries, plays a central role in this heightened risk. Inflammation, a complex biological response to harmful stimuli, is a double-edged sword. While acute inflammation is a crucial defense mechanism against infection and injury, chronic, low-grade inflammation can wreak havoc on the body, contributing to a wide range of diseases, including CKD and atherosclerosis. In CKD, the kidneys undergo a series of pathological changes, including glomerulosclerosis, tubulointerstitial fibrosis, and vascular damage. These changes disrupt the delicate balance of the kidney's filtration and regulatory functions, leading to the accumulation of waste products and fluid imbalances in the body. Inflammation is a key driver of these pathological changes. A constellation of factors contributes to the chronic inflammatory state in CKD. An imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant defenses leads to oxidative stress, a state of molecular mayhem that damages cellular components and triggers inflammatory pathways. The mitochondria, the powerhouses of the cell, are essential for energy production. In CKD, mitochondrial dysfunction leads to increased production of ROS, overwhelming the cell's antioxidant defenses. This dysfunction is exacerbated by the accumulation of uremic toxins, which further impair mitochondrial function. The body has a natural defense system against ROS, composed of antioxidant enzymes such as superoxide dismutase (SOD), catalase, and glutathione peroxidase. In CKD, the activity of these enzymes is reduced, compromising the body's ability to neutralize ROS. Activated immune cells, such as neutrophils and macrophages, generate ROS as part of their defense mechanism. In CKD, the persistent inflammation leads to chronic activation of these cells, further fueling the oxidative fire. This oxidative stress further activates NF-κB, a master regulator of inflammation, creating a vicious cycle of inflammation and kidney damage. The activation of NF-κB leads to the expression of more pro-inflammatory cytokines and chemokines, which in turn attract more immune cells, generating more ROS, and further amplifying the inflammatory response. The accumulation of waste products in the blood, known as uremic toxins, can activate immune cells and promote inflammation. These toxins, which are normally excreted by the kidneys, can bind to and activate various receptors on immune cells, leading to the release of pro-inflammatory cytokines and chemokines. Uremic toxins can activate pattern recognition receptors (PRRs) on immune cells, such as Toll-like receptors (TLRs) and NOD-like receptors (NLRs). These receptors are normally involved in recognizing pathogens and initiating immune responses. However, in CKD, the activation of these receptors by uremic toxins leads to inappropriate and chronic inflammation. Some uremic toxins, such as indoxyl sulfate and p-cresyl sulfate, have been shown to directly activate NF-κB, further amplifying the inflammatory response. These toxins can enter the cells and interact with intracellular signaling pathways, leading to the activation of NF-κB and the expression of pro-inflammatory genes. These signaling molecules, such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), amplify the inflammatory response, attracting immune cells and perpetuating the cycle of inflammation and tissue damage. Mesangial Cells are located in the glomerulus, the filtering unit of the kidney. In CKD, mesangial cells become activated and produce pro-inflammatory cytokines, contributing to glomerular damage and sclerosis. Tubular Epithelial Cells line the tubules of the kidney, which are responsible for reabsorbing

essential nutrients and excreting waste products. In CKD, tubular epithelial cells also become activated and produce pro-inflammatory cytokines, contributing to tubular damage and interstitial fibrosis. Infiltrating Immune Cells persistent inflammation in CKD attracts various immune cells, such as macrophages, neutrophils, and T cells, to the kidneys. These cells release pro-inflammatory cytokines, further amplifying the inflammatory response. Fibrosis is the excessive accumulation of extracellular matrix proteins, leading to scarring and loss of function. Pro-inflammatory cytokines promote fibrosis by stimulating the production of collagen and other matrix proteins by fibroblasts. Apoptosis is a programmed cell death process. Pro-inflammatory cytokines can induce apoptosis of kidney cells, contributing to the loss of functional nephrons. Pro-inflammatory cytokines can impair the function of blood vessels, leading to reduced blood flow to the kidneys and further damage. Inflammation and kidney damage are intertwined in a vicious cycle. The initial insult to the kidneys, whether from diabetes, hypertension, or other causes, triggers an inflammatory response. This inflammation, in turn, exacerbates kidney damage, leading to further inflammation, and so on. This self-perpetuating cycle contributes to the progressive decline in kidney function that characterizes CKD. Inflammation not only drives kidney damage but also acts as a bridge between CKD and atherosclerosis. The chronic inflammatory state in CKD spills over into the systemic circulation, affecting the vasculature and promoting the development of atherosclerosis. Atherosclerosis is a chronic inflammatory disease of the arteries characterized by the buildup of plaque within the arterial walls. This plaque, composed of cholesterol, immune cells, and other debris, narrows and hardens the arteries, impairing blood flow and increasing the risk of cardiovascular events. Inflammation plays a pivotal role in all stages of atherosclerosis, from the initial endothelial dysfunction to the rupture of vulnerable plaques. In CKD, the heightened inflammatory state accelerates the atherosclerotic process, increasing the risk of cardiovascular complications. The endothelium, the inner lining of blood vessels, plays a crucial role in regulating vascular tone and preventing platelet aggregation. In CKD, the chronic inflammation damages the endothelium, impairing its function and promoting the adhesion of immune cells and platelets to the vessel wall. This initiates the atherosclerotic process. CKD is often associated with dyslipidemia, an abnormal level of lipids in the blood. This includes elevated levels of LDL cholesterol ("bad" cholesterol) and triglycerides, and decreased levels of HDL cholesterol ("good" cholesterol). These lipid abnormalities promote the formation of foam cells, which are macrophages that have engulfed cholesterol and become laden with fat. Foam cells are a key component of atherosclerotic plaques. As mentioned earlier, oxidative stress is a major contributor to inflammation in CKD. This oxidative stress also plays a crucial role in atherosclerosis by oxidizing LDL cholesterol, making it more likely to be taken up by macrophages and form foam cells. Nuclear factor kappa B (NF-κB) is a family of transcription factors that act as master regulators of inflammation. NF-κB is like a conductor of an orchestra, coordinating the expression of numerous genes involved in inflammation, immunity, and cell survival. In CKD, NF-κB activation is like an orchestra playing a cacophony of inflammatory music. TNF-α, IL-6, IL-1β, and others, which amplify the inflammatory response and contribute to kidney damage. Chemokines signaling molecules attract immune cells to the site of inflammation, further exacerbating the inflammatory response. Adhesion Molecules, expressed on the surface of endothelial cells, facilitate the adhesion and migration of immune cells into the kidney tissue. In atherosclerosis, NF-κB activation is like a conductor leading the orchestra to play a symphony of plaque formation and instability. Adhesion Molecules such as VCAM-1 and ICAM-1, which promote the adhesion of monocytes (a type of white blood cell) to the endothelium, initiating the formation of atherosclerotic plaques. Pro-inflammatory Cytokines

which further activate immune cells and promote the recruitment of more monocytes and other inflammatory cells to the plaque. Matrix Metalloproteinases (MMPs) enzymes degrade the extracellular matrix, weakening the fibrous cap of the plaque and making it more prone to rupture. Plaque rupture can lead to the formation of blood clots, which can block arteries and cause heart attacks or strokes. Given the central role of inflammation in CKD and its associated cardiovascular complications, targeting inflammation is a promising therapeutic strategy. Healthy lifestyle choices, such as a balanced diet, regular exercise, and stress management, can help reduce inflammation. High sodium intake can exacerbate fluid retention and increase blood pressure, both of which can worsen kidney function and inflammation. While protein is essential for the body, excessive protein intake can increase the workload on the kidneys and contribute to the buildup of uremic toxins. Fruits and vegetables are rich in antioxidants, which can help combat oxidative stress. They also provide fiber, which can help lower cholesterol levels and improve gut health, further reducing inflammation. Omega-3 Fatty Acids healthy fats, found in fatty fish, flaxseed, and walnuts, have anti-inflammatory properties and can help protect against cardiovascular disease. Physical activity has numerous benefits, including reducing inflammation, improving blood pressure and cholesterol levels, and promoting weight loss. Chronic stress can contribute to inflammation. Relaxation techniques such as meditation, yoga, and deep breathing can help manage stress and reduce inflammation. Statins cholesterol-lowering medications also have anti-inflammatory effects. They can help reduce inflammation in the blood vessels and slow the progression of atherosclerosis. Nonsteroidal anti-inflammatory drugs (NSAIDs) can help reduce inflammation and pain. However, they should be used with caution in CKD patients, as they can worsen kidney function. Renin-Angiotensin-Aldosterone System (RAAS) Inhibitors medications, including ACE inhibitors and ARBs, are commonly used to treat hypertension and CKD. They also have anti-inflammatory effects by reducing the production of angiotensin II, a hormone that promotes inflammation and fibrosis. Anti-cytokine Therapies target specific pro-inflammatory cytokines, such as TNF-α and IL-6. They can help reduce inflammation and slow the progression of kidney damage. However, they can also have significant side effects and are not yet widely used in CKD. NF-κB Inhibitors therapies aim to directly block the activation of NF-κB, thereby reducing the expression of pro-inflammatory genes. Several NF-κB inhibitors are under development and show promise for treating inflammatory diseases, including CKD. However, further research is needed to evaluate their efficacy and safety in CKD patients.[11,12]

Nuclear factor kappa B (NF-κB) isn't just a string of complex-sounding letters, it's a critical player in the drama of inflammation unfolding within our bodies. Imagine it as a powerful conductor orchestrating a complex symphony of cellular responses. In healthy individuals, this conductor keeps the music balanced and harmonious, ensuring that inflammation, a vital defense mechanism, is initiated when needed and then gracefully subsides. However, in the context of chronic kidney disease (CKD), the conductor becomes overzealous, leading to a cacophony of inflammatory signals that contribute to the relentless progression of kidney damage and cardiovascular complications. NF-κB is a family of proteins that act as transcription factors, meaning they regulate the expression of genes. They are like master switches, turning genes on or off in response to various signals. In their resting state, these molecular maestros are held captive in the cell's cytoplasm, bound to inhibitory proteins called IκBs. These IκBs act as molecular handcuffs, preventing NF-κB from entering the cell's control center, the nucleus, and wreaking havoc. However, when the cell senses danger – be it from invading pathogens, injury, or internal distress signals like oxidative stress – a cascade of molecular events is set in motion, ultimately freeing NF-κB from its cytoplasmic prison. This liberation allows NF-κB to enter the nucleus, where it binds to specific DNA

sequences and activates the transcription of numerous genes involved in inflammation, immunity, and cell survival. In CKD, the kidneys become a stage for chronic inflammation, with NF-κB taking center stage as the conductor of this inflammatory orchestra. A multitude of factors contribute to the persistent activation of NF-κB in CKD, turning the harmonious symphony into a dissonant cacophony. As the kidneys fail, they lose their ability to efficiently filter waste products from the blood. These accumulated waste products, known as uremic toxins, act as irritants, triggering inflammation throughout the body. Some of these toxins directly activate NF-κB, further fueling the inflammatory fire. Imagine a cell as a delicate ecosystem, where a balance between oxidants and antioxidants is crucial for maintaining harmony. In CKD, this balance is disrupted, leading to a state of oxidative stress. This molecular imbalance damages cellular components and triggers inflammatory pathways, further activating NF-κB and perpetuating the cycle of inflammation and kidney damage. Pro-inflammatory Cytokines signaling molecules are like the messengers of inflammation, amplifying the inflammatory response and attracting immune cells to the site of damage. In CKD, these cytokines are produced in excess, creating a feedback loop that sustains the inflammatory response and contributes to kidney damage. The persistent activation of NF-κB in CKD has a devastating impact on the body. NF-κB orchestrates the production of various inflammatory mediators that directly damage the kidneys. These mediators recruit immune cells, promote fibrosis (scarring), and induce apoptosis (programmed cell death) of kidney cells, leading to progressive loss of kidney function. The inflammatory symphony in CKD doesn't remain confined to the kidneys, it spills over into the systemic circulation, affecting the blood vessels and promoting the development of atherosclerosis. NF-κB activation in the blood vessels leads to the recruitment of immune cells and the formation of atherosclerotic plaques, increasing the risk of heart attacks and strokes. The inflammatory signals orchestrated by NF-κB extend beyond the kidneys and blood vessels, contributing to a state of chronic, low-grade inflammation throughout the body. This systemic inflammation can lead to fatigue, malnutrition, and other complications in CKD patients, further diminishing their quality of life. Recognizing the central role of NF-κB in the pathogenesis of CKD, researchers are actively exploring strategies to quiet this overactive conductor and restore harmony to the inflammatory response. Adopting a healthy lifestyle can significantly dampen the inflammatory symphony. A balanced diet rich in antioxidants, regular exercise, and stress management techniques can all help reduce inflammation and NF-κB activation. Certain medications, such as statins (cholesterol-lowering drugs) and RAAS inhibitors (blood pressure medications), have anti-inflammatory properties and can help modulate NF-κB activity. Scientists are developing new therapies that directly target NF-κB or the inflammatory pathways it controls. These include anti-cytokine therapies, which block the action of specific pro-inflammatory cytokines, and NF-κB inhibitors, which directly inhibit the activity of NF-κB.[13,14]

Propolis, a sticky, resinous substance collected by honeybees from tree buds, sap flows, and other botanical sources, has been prized for its medicinal properties since ancient times. Often referred to as "bee glue," propolis serves as a natural sealant and sterilizing agent within the beehive, protecting the colony from microbial invaders and environmental stressors. This remarkable substance, with its complex chemical composition and diverse biological activities, has captured the attention of scientists and healthcare practitioners alike, particularly for its potent anti-inflammatory properties. Propolis is a complex mixture of over 300 identified compounds, including flavonoids, phenolic acids, terpenoids, and amino acids. This rich tapestry of bioactive molecules contributes to its diverse pharmacological activities, making it a valuable natural remedy for a wide range of ailments. Flavonoids potent antioxidants are abundant in propolis, contributing to its anti-

inflammatory, antioxidant, and anticancer properties. Flavonoids can scavenge free radicals, protect cells from oxidative damage, and modulate inflammatory signaling pathways. Phenolic Acids compounds also possess antioxidant and anti-inflammatory activities. They can inhibit the production of pro-inflammatory cytokines and enzymes, reducing inflammation and protecting tissues from damage. Terpenoids diverse compounds contribute to the antimicrobial and anti-inflammatory properties of propolis. They can inhibit the growth of bacteria, fungi, and viruses, and also modulate immune responses. The anti-inflammatory effects of propolis have been largely attributed to its ability to modulate the NF-κB signaling pathway. NF-κB, a family of transcription factors, plays a central role in regulating inflammatory responses. When activated, NF-κB orchestrates the expression of numerous genes involved in inflammation, immunity, and cell survival. Propolis, like a skilled conductor, can dampen the inflammatory symphony orchestrated by NF-κB. IκBα is an inhibitory protein that keeps NF-κB in its inactive state. Propolis can prevent the degradation of IκBα, keeping NF-κB locked in its cytoplasmic prison and preventing it from activating inflammatory genes. Even if NF-κB is liberated from its inhibitory handcuffs, propolis can still prevent it from entering the nucleus, the cell's control center. This prevents NF-κB from binding to DNA and activating the transcription of inflammatory genes. Propolis can directly interfere with the ability of NF-κB to bind to DNA, preventing it from activating the expression of inflammatory genes. In vitro and in vivo studies have demonstrated the potential benefits of propolis in attenuating kidney injury and improving renal function. Propolis can reduce the production of pro-inflammatory cytokines and chemokines, dampening the inflammatory response in the kidneys. The antioxidant properties of propolis help protect kidney cells from oxidative damage, reducing inflammation and fibrosis. Propolis can inhibit the production of collagen and other extracellular matrix proteins, reducing the accumulation of scar tissue in the kidneys. Propolis has been shown to improve renal blood flow and glomerular filtration rate, enhancing the kidney's ability to filter waste products from the blood. The anti-inflammatory effects of propolis extend beyond the kidneys. Propolis can help reduce inflammation in the blood vessels, protect against atherosclerosis, and improve heart health. Propolis can help reduce inflammation in the airways, alleviate symptoms of asthma and bronchitis, and boost immune function. Propolis can help reduce inflammation in the gut, alleviate symptoms of inflammatory bowel disease, and protect against ulcers. Propolis can help reduce inflammation and promote healing in various skin conditions, such as eczema, psoriasis, and acne.[15,16]

Chronic kidney disease (CKD) is a debilitating condition characterized by the gradual loss of kidney function. It affects millions worldwide, casting a long shadow over their lives and placing a significant burden on healthcare systems. One of the hallmarks of CKD is chronic inflammation, a relentless fire that fuels the progression of kidney damage and contributes to the development of cardiovascular complications. In this study, we delved into the intricate world of inflammation in CKD, focusing on a key player known as nuclear factor kappa B (NF-κB). NF-κB is a master regulator of inflammation, orchestrating the expression of numerous genes involved in the inflammatory response. In CKD, NF-κB is like an overzealous conductor, leading to a cacophony of inflammatory signals that damage the kidneys and promote cardiovascular disease. Our quest was to find a way to tame this inflammatory conductor, to restore harmony to the cellular orchestra. We turned to propolis, a natural resinous substance collected by bees, renowned for its anti-inflammatory properties. Propolis is a treasure trove of bioactive compounds, including flavonoids, phenolic acids, and terpenoids, which possess a remarkable ability to modulate the inflammatory response. To investigate the effects of propolis extract on NF-κB activation in CKD, we employed a rat model of CKD known as unilateral ureteral obstruction (UUO). UUO involves the surgical blockage of one of the ureters, the

tubes that carry urine from the kidneys to the bladder. This obstruction mimics the physiological changes that occur in human CKD, leading to the accumulation of waste products, inflammation, and fibrosis in the affected kidney. The UUO model is a valuable tool for studying the mechanisms of CKD progression and evaluating the efficacy of potential therapeutic interventions. It allows researchers to explore the complex interplay of factors that contribute to kidney damage and to test new therapies in a controlled environment. Our study revealed that propolis extract significantly attenuated NF-κB activation in CKD rats. This remarkable finding suggests that propolis extract can effectively dampen the inflammatory response in CKD, potentially slowing the progression of kidney damage and reducing the risk of cardiovascular complications. The anti-inflammatory effects of propolis extract can be attributed to its diverse bioactive compounds, which act in concert to modulate the NF-κB pathway. IκBα is an inhibitory protein that keeps NF-κB in its inactive state. Propolis extract can prevent the degradation of IκBα, keeping NF-κB locked away in the cytoplasm and preventing it from activating inflammatory genes. Even if NF-κB is released from its inhibitory shackles, propolis extract can still prevent it from entering the nucleus, the cell's control center. This prevents NF-κB from binding to DNA and activating the transcription of inflammatory genes. Propolis extract can directly interfere with the ability of NF-κB to bind to DNA, preventing it from turning on the expression of inflammatory genes. Our findings suggest that propolis extract may hold promise as an adjunctive therapy for CKD, particularly in the context of surgical interventions. Surgical procedures can trigger an inflammatory response, which may exacerbate CKD progression. Propolis extract, with its ability to attenuate NF-κB activation, may help to mitigate the inflammatory response associated with surgical interventions, thereby improving outcomes in CKD patients undergoing surgery.[17,18]

Chronic kidney disease (CKD) is a complex and multifaceted condition, often requiring a multi-pronged approach to management. While traditional therapies such as lifestyle modifications and pharmacological interventions form the cornerstone of CKD management, there is a growing recognition of the need for adjunctive therapies that can complement these existing approaches and address specific aspects of the disease process. Our study, demonstrating the potent anti-inflammatory effects of propolis extract in a rat model of CKD, has significant implications for the development of such adjunctive therapies. Propolis extract, with its ability to attenuate NF-κB activation, may hold the key to mitigating the inflammatory burden in CKD, particularly in the context of surgical interventions. For individuals with CKD, surgical procedures can be a double-edged sword. While surgery may be necessary to address various complications or comorbidities associated with CKD, it also poses a significant risk of exacerbating the disease process. Surgery, by its very nature, is a traumatic event for the body. It triggers a complex inflammatory response, characterized by the release of pro-inflammatory cytokines, chemokines, and other mediators. This inflammatory response is essential for wound healing and tissue repair. However, in individuals with CKD, the pre-existing chronic inflammation and impaired immune function can amplify the surgical inflammatory response, potentially leading to adverse outcomes. In CKD patients undergoing surgery, the inflammatory cascade can be particularly detrimental. CKD is characterized by a chronic inflammatory state, driven by factors such as oxidative stress, uremic toxins, and pro-inflammatory cytokines. This pre-existing inflammation creates a primed environment for an exaggerated response to surgical trauma. CKD can impair immune function, making individuals more susceptible to infections and complications. This impaired immune response can also contribute to a prolonged and dysregulated inflammatory response following surgery. Surgical procedures can further increase oxidative stress, exacerbating the inflammatory response and contributing to kidney damage. Surgical stress can worsen kidney function,

leading to further accumulation of uremic toxins. These toxins can further activate inflammatory pathways and contribute to complications. Our study provides compelling evidence that propolis extract can effectively attenuate NF-κB activation, a key driver of inflammation in CKD. This suggests that propolis extract may have a protective role in mitigating the exaggerated inflammatory response associated with surgical interventions in CKD patients. Inflammation can contribute to various post-surgical complications, such as infection, delayed wound healing, and organ dysfunction. Propolis extract, by reducing inflammation, may help to prevent or mitigate these complications. The inflammatory response to surgery can worsen kidney function, potentially accelerating the progression of CKD. Propolis extract, by attenuating inflammation, may help to protect the kidneys from further damage. By reducing inflammation and protecting kidney function, propolis extract may contribute to improved overall surgical outcomes in CKD patients. The potential benefits of propolis extract in CKD management extend beyond its anti-inflammatory effects. The antioxidant properties of propolis extract can help protect kidney cells from oxidative damage, further reducing inflammation and fibrosis. Propolis extract can inhibit the production of collagen and other extracellular matrix proteins, reducing the accumulation of scar tissue in the kidneys. Propolis extract has been shown to improve renal blood flow and glomerular filtration rate, enhancing the kidney's ability to filter waste products from the blood.[19,20]

5. Conclusion

This study investigated the effects of propolis extract on NF-κB activation in a rat model of CKD. The results demonstrated that propolis extract significantly attenuated NF-κB activation in CKD rats, suggesting its potential as an adjunctive therapy for CKD management. The UUO method was used to induce CKD in rats. UUO is a well-established model of CKD that mimics the pathophysiological changes observed in human CKD, including inflammation, fibrosis, and oxidative stress.

6. References

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