

Anti-inflammatory activity of date palm seed by downregulating interleukin-1b, TGF-b, cyclooxygenase-1 and -2: A study among middle age women

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Anti-inflammatory activity of date palm seed by downregulating interleukin-1 β , TGF- β , cyclooxygenase-1 and -2: A study among middle age women



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ABSTRACT

The prevalence of degenerative diseases increases with age. Furthermore, various factors tend to trigger cells injury, thereby, causing inflammation. This study, therefore, aims to examine the anti-inflammatory mechanisms of steeping date seeds in middle age women. This is a quasi-experimental design with a pre-and post-test approach used to evaluate the anti-inflammatory effect of 2.5 g of steeped of date palm seed, consumed by 30 healthy middle-aged women per day (in 250 mL water) for 14 days. The final numbers (22 subjects) of recruited women were included in the statistical analysis. Their level of IL-1 β , TGF- β , IL-6, TNF- α , IL-12, COX-1, COX-2, and PGE2 were determined using ELISA. The results showed that the expression of IL-1 β , TGF- β , COX-8 and COX-2 in women significantly decreased after consuming date palm seed. Steeped of date seed acts as an anti-inflammatory by downregulating the expression of key proinflammatory mediators.

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1. Introduction

Chronic and degenerative diseases in middle-aged women such as cancer, cardiovascular, and metabolic diseases are increasing. These diseases are the leading cause of death and disability disorders in developing countries. Kim et al., 2018 showed that large changes in behavior patterns due to the decreasing consumption of vegetables and fruits, which plays an important role in increasing the incidence of chronic and degenerative diseases. Bioactive compounds in fruits and vegetables such as polyphenols, flavonoids, anthocyanins, micronutrients, minerals and vitamins, have antioxidant and anti-inflammatory activities (Sofi and Dinu, 2016). Therefore, they possess preventive and therapeutic potencies against diseases (Aguilera et al., 2016; Wang et al., 2016).

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Date palm (*Phoenix dactylifera L.*) seed is one of the rich source of polyphenols and flavonoids (Djaoudene et al., 2019). It has been extensively investigated for pharmacological activities such as anti-inflammatory (Saryono et al., 2018), immuno-stimulant (Saryono et al., 2019), antidiabetic (El-Fouhail et al., 2010), antibacterial (Chinelo et al., 2019), antiviral (Jassim and Naji, 2010), and antioxidant (Bouhlali et al., 2015; Djaoudene et al., 2019; Habib and Ibrahim, 2011; Platat et al., 2019). In previous studies, the date palm seeds were proven to work as anti-inflammatory (Saryono et al., 2019; Saryono et al., 2019b, 2019a), and antiatherogenic food substance (Saryono et al., 2017). Recent study showed that various compounds act as anti-inflammatory by metabolomic approach (Abdul-Hamid et al., 2019). Anti-inflammatory effect of date seeds in human has not been adequately studied, especially in middle-aged women. This study, therefore, aims to examine the anti-inflammatory mechanisms of steeped date palm seed in middle-aged women.

2. Material and methods

2.1. Study design and participants

This is a quasi-experimental design with a pre and post-test approach. Data were obtained from a total of 30 randomly selected

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middle-aged women living at Gununglurah, Cilongok District, Banyumas. The inclusion criteria were women between the age of 45–60 years, with no history of metabolic disease by measuring their blood glucose and total cholesterol levels. These women live in farming community and perform the domestic tasks of housewives. In this study, each subject received a single dose of 2.5 g seed powder per day for 14 days. The seed powder was consumed using 250 mL of boiling water. Subjects signed an informed consent before participating in this study, which was conducted after obtaining approval of ethical clearance from the medical research ethics committee of the Faculty of Medicine University of Negeri Sebelas Maret Surakarta, number: 541/IV/HREC/2019.

2.2. Preparation of date seeds powder

Deglet Nour dates collected from Tunisia, were obtained from a market at Purwokerto, Banyumas Regency, Indonesia. Seeds were manually separated from the date flesh, cleaned with water, selected, and dried for one day in sunlight. The dried seeds were roasted at medium temperature, crushed in a blender and filtered to obtain a fine powder.

2.3. Measurement pro-inflammatory mediators

A 3 mL blood sample was taken through the median cubital vein before and after treatment. The level of IL-1 β , TNF- α , IL-6, IL-12, TGF- β , COX-1, COX-2, and prostaglandin E2 were examined by ELISA kit (BT Laboratories, Shanghai) based on the manufacturer's protocol, using an ELISA machine Reader (Labotrone, Germany).

2.4. Statistical analysis

The mean score was compared before and after treatment with all data presented as mean \pm SEM. Differences in scores before and after treatment were analyzed using the student *t*-test. The graph pad software (GraphPad Prism, San Diego, CA) was used to analyze statistical and graphical data, with a *p* value < 0.05 considered significant.

3. Results

3.1. Characteristics of participants

Thirty subjects were initially recruited, however, eight were unable to complete the treatment. Therefore, a total of 22 subjects were included in the statistical analysis. The age of subjects ranged was from 51 to 57 years, with a healthy weight status and an average body mass index of 24 kg/m², blood glucose below 140 mg/dL, and total cholesterol below 200 mg/dL (Table 1).

3.2. Expression of proinflammatory cytokines

The expression of IL-1 β and TGF- β reduced significantly after the consumption of date palm seeds in the post-treatment stage, while TNF- α , IL-12, and IL-6 did had no significant change

Table 1
Respondent characteristic.

Variable	Ranges	Mean
Age (years)	51-57	55
Blood pressure		
Systole (mmHg)	100-175	135
Diastole (mmHg)	65-100	93
Body mass index (kg/m ²)	23.5-24.5	24

(Fig. 1). This result suggests the date palm seed reduces IL-1 β and TGF- β produced in healthy middle-aged women.

3.3. The role of dates palm seed on cyclooxygenase pathway

The cyclooxygenase (COX) pathway is important in arachidonic acid metabolism related to the inflammatory process (Levick et al., 2007). Therefore, the expression of COX-1, COX-2, as well as prostaglandin E2 level, showed that these mediators are important markers on the COX pathway. A significant decrease in the level of COX-1, COX-2 and PGE2 was observed in the post-treatment stage compared with pre-treatment of steeped of date palm seeds (Fig. 2). Interestingly, the expression of COX-2 substantially decreased (*p* < 0.001), which indicates that the inducible expression of COX-2 in middle-aged women was decreased by consumption of date palm seeds.

4. Discussion

Several studies have been conducted to examine the potential of date palm seeds as an anti-inflammatory (Barakat et al., 2020) in vitro and in vivo (Djaoudene et al., 2019; Maqsood et al., 2020; Saryono et al., 2018). However, no anti-inflammatory activity studies have been conducted on date palm seeds in humans, women middle age. This study, found that the expression of IL-1 β , TGF- β , COX-1 and COX-2 decreased after the administration of date palm seeds to middle age women.

Studies showed that the elderly and middle age women experience an increase in free radicals due to various factors such as aging, food, pollution, and excess activity. Free radicals in the body causes oxidation in normal cells, thereby, leading to inflammation and diseases (Sies, 2018; Suleman, 2018). Lymphocytes and other immune cells produce cytokines such as IL-1 β and TGF- β when there are inflammatory stimuli.

Interleukin-1 β is highly elevated in chronic diseases such as obesity, osteoarthritis, and gout (Dinarello, 2011). IL-1 β affects lymphocytes and macrophages, induces the formation of prostaglandins, colony stimulating factors and other cytokines (Wojdasiewicz et al., 2014). IL-1 β expression is stimulated by various stimuli including sterile stimulus through the formation of inflammasomes (Shi et al., 2015). Since middle age women have developed accumulated agents capable of activating the inflammasome formation, such as cholesterol and uric acid (Qin et al., 2014), it is therefore, important to determine the ability of the date palm seed to reduce the expression of IL-1 β . Another interesting aspect is to reveal the role of date palm seeds in inhibiting IL-1 β expression, which is further investigated in future.

Although TGF- β was initially called as anti-inflammatory cytokines, it also induces inflammatory Th17 cells differentiation (Yoshimura et al., 2010). Although the role TGF- β in inflammation remains unclear, the date palm seed showed its ability to decrease.

Cyclooxygenase pathway has been well-established for their role in inflammation (Hanna and Hafez, 2018). Conversion of phospholipids to arachidonic acid is mediated by the enzyme cyclooxygenase. COX-1 is constitutively expressed and plays a role in the protection of the gastric mucosa. COX-2 is expressed inducibly by an inflammatory stimulus (Urban, 2000). Furthermore, various stimuli tend to induce COX-2 expression, which tends to occur with increasing age. In this study, COX-2 expression greatly decreased after the administration of date palm seeds to middle age women. Both enzymes induce the conversion of arachidonic acid into prostaglandins, e.g. PGE2 which plays a role in the vasodilation of blood vessels and increased vascular endothelial permeability (Kawahara et al., 2015). The expression of both COX

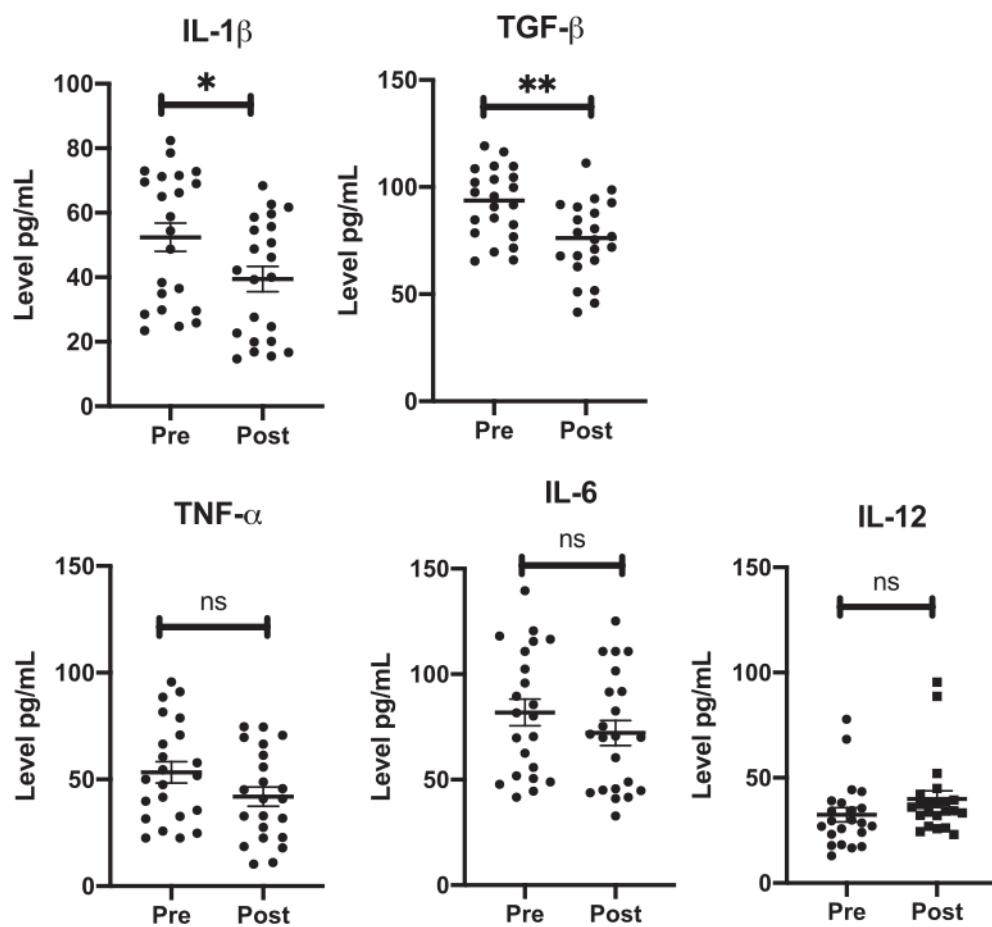


Fig. 1. Treatment date palm seed decreases IL-1 β and TGF- β expression. The cytokines levels were measured at day 0 (pre-treatment) and day 15 (post-treatment) by enzyme-linked immunosorbent assay (ELISA). Mean \pm SE are presented ($n = 22$). Asterisks indicate student *t*-test significance values; ** $P < 0.01$, * $P < 0.05$. ns = not significant.

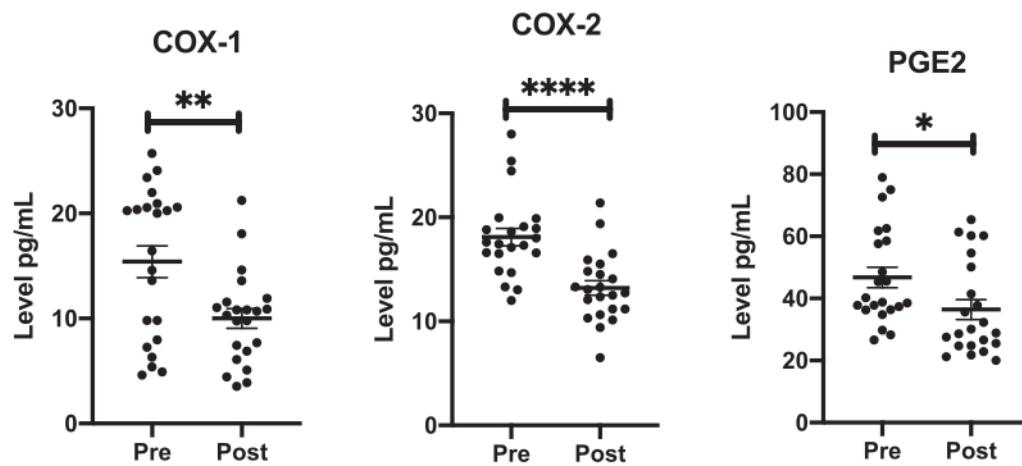


Fig. 2. Treatment date palm seed decreases COX-1, COX-2, PGE2 level. The enzyme/mediator levels were measured at day 0 (pre-treatment) and day 15 (post-treatment) by enzyme-linked immunosorbent assay (ELISA). Mean \pm SE are shown ($n = 22$). Asterisks indicate student *t*-test significance values; ** $P < 0.01$, *** $P < 0.001$.

enzymes is inhibited by date palm seeds, with a decrease in PGE2 production.

This study is limited to high of dropping out because the subject did not complete the consumption of date palm seed powder as assigned. In addition, the food consumed by the subjects and likely to affect the level of proinflammatory mediators in the human body were not recorded.

Date palm seeds may work as an anti-inflammatory and improve the performance of the immune system (Saryono et al., 2019). Rahmani et al., 2014 stated that it works to suppress NF- κ B, COX-1 and COX-2 enzymes, thereby, decreasing proinflammatory mediators. The anti-inflammatory activity of the aqueous extract of date palm seeds is related to components of polyphenols such as caffeoyl hexoside, 5-O-caffeyl shikimic acid isomers, hydrocaffeic acid, and isorhamnetin (Thouri et al., 2017; John and Shahidi, 2019). This ingredient has also been proven safe for liver and kidney (El Fouhil et al., 2011), therefore, it can be consumed regularly by middle age women to maintain health status, improve immune systems, and prevent chronic diseases. Clinical implications obtained from this study shows that the physicians may suggest the steeped of palm seed powder as a functional beverage.

5. Conclusion

Dates seeds can act as an anti-inflammatory by reducing interleukin-1 β , TGF- β , cyclooxygenase-1 and -2 expression. Regular consumption of date palm seeds increases the body's immunity and prevent chronic diseases.

Declaration of Competing Interest

The authors declared that there is no conflict of interest.

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Authors' contributions

SY, W, AI conceived and designed the experiments; SY, W, AI performed the experiments; SM analyzed the data; SY, W, AI contributed chemicals/reagents/materials/analysis tools; SY and SM wrote the paper. The authors read and approved the final manuscript.

Appendix

Availability of Data and Materials

All data generated or analyzed during this study are included in this manuscript. Raw data are available from the corresponding author on a reasonable request.

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