J Herbmed Pharmacol. 2019; 8(3): 212-217.

http://www.herbmedpharmacol.com doi: 10.15171/jhp.2019.31

Journal of Herbmed Pharmacology

Dietary supplementation of *Phoenix dactylifera* L. seeds decreases pro-inflammatory mediators in CCl4-induced rats

Saryono Saryono[1], Agis Taufik[1], Atikah Proverawati[2], Ferry Efendi[3*]

[1]School of Nursing, Faculty of Health Sciences, University of Jenderal Soedirman, Purwokerto, Indonesia
[2]School of Nutrition, Faculty of Health Sciences, University of Jenderal Soedirman, Purwokerto, Indonesia
[2]Faculty of Nursing, Universitas Airlangga, Surabaya, Indonesia

ARTICLE INFO

Article Type:
Original Article

Article History:
Received: 18 September 2018
Accepted: 7 March 2019

Keywords:
TNF-α
GSH
IFN-γ
Date seed
Anti-inflammation

ABSTRACT

Introduction: Low immunity causes the body to become more easily infected, resulting in inflammation. If the immune system is functioning properly, this inflammation will end in healing. The immune system has a protective role in the body, and its anti-inflammatory role is vital. During trauma, the initial immune response is marked by inflammation. The use of date-seed extract, although not steeped date seeds, has been studied as an anti-inflammatory agent. This study is aimed at demonstrating the anti-inflammatory effect of steeped date seeds (*Phoenix dactylifera* L.) in rats with CCl_4-induced inflammation.

Methods: This experiment included a pre- and post-test with control group design. Male Wistar rats (approximately 2–3 months of age, ranging in weight from 150 to 200 g) were assigned to the following groups: negative control (NC), positive control (PC), T1 treatment dose 1 g/kg, T3 treatment dose 3 g/kg, T5 treatment dose 5 g/kg, and healthy control (HC). Groups 1–5 were subjected to CCl_4 induction at a single dose of 2 mL/kg before treatment. The levels of tumor necrosis factor (TNF-α), glutathione (GSH), and gamma interferon (IFN-γ) were compared in groups using one-way analysis of variance (ANOVA), followed by a least significant difference (LSD) post hoc test for comparisons between means.

Results: Levels of tumor necrosis factor (TNF-α), GSH, and IFN-γ were significantly different among the HC and treatment groups after CCl_4 induction. After 14 days of steeped date-seed treatment, TNF-α decreased, but GSH and IFN-γ levels increased significantly (P = 0.001).

Conclusion: Administration of steeped date seeds at a dose of 5 g/kg can increase GSH and IFN-γ, and decrease TNF-α, the strongest inflammatory marker in CCl_4-induced rats. The findings of this study indicate that date-seed supplementation can support body immunity by regulating pro-inflammatory mediators.

Implication for health policy/practice/research/medical education:
This study revealed that date seeds can be considered as a supplementary and natural source of immune-stimulant to maintain health.

Please cite this paper as: Saryono S, Taufik A, Proverawati A, Efendi F. Dietary supplementation of *Phoenix dactylifera* L. seeds decreases pro-inflammatory mediators in CCl_4-induced rats. J Herbmed Pharmacol. 2019;8(3):212-217. doi: 10.15171/jhp.2019.31.

Introduction

The human body is particularly vulnerable to external antigen exposure from viruses, bacteria, and fungi (infectious diseases), as well as from abnormal growths such as tumors, and degenerating cells. Degenerative diseases and exposure to microorganisms can suppress immunity. Dietary habits, such as fast food consumption, can also trigger a decline in the immune system. Fast foods contain many free radicals, and increased free radicals in the body can damage lipids, proteins and DNA (1–3). Moreover, free radicals from toxic compounds such as carbon tetrachloride (CCl_4) can decrease the body's immune system ability (4,5). Although the body has a complex immune defense system, sometimes it is unable to deal with certain toxins.

Some infectious diseases can be prevented by enhancing the body's immune system and reducing exposure to toxic compounds. These efforts may include a local immunostimulant. Various efforts have been made to improve the response of the body's immune system; e.g., the use of immunostimulant drugs. However, these efforts have been ineffective because many chemical compounds in drugs cause side effects, while many medicines also have immunosuppressive effects. Therefore, herbal therapy

***Corresponding author**: Ferry Efendi, Email: ferry-e@fkp.unair.ac.id

is considered as a solution for this issue, particularly the use of date seeds. Date seeds contain many phenolic and antioxidant compounds that are believed to stimulate the body's immune system (6). The chlorogenic acid, pelargonin and ferulic acid found in dates can significantly reduce the number of immunoglobulin E (IgE) cells in the allergic response (2).

The consumption of dates in Indonesia, the largest Muslim country in the world, is very high; however, date seeds are usually thrown away. Date seeds contain many active compounds in the form of antioxidants, such as phenolics, which may reduce free-radical levels by virtue of their anti-inflammatory and immunostimulant activities (7,8). The phenolic content of date seeds includes protocatechuic acid, p-hydroxybenzoic, gallic acid, vanillic acid, caffeine acid, p-coumaric acid, m-coumaric, and o-coumaric (9). The content of phenolic acid as antioxidant reduces free radicals induced by both cancer and anti-cancer treatment. In addition, date seeds contain quercetin, which can increase the synthesis of T and B lymphocytes (10,11). A date-seed diet has been found to improve the performance and response as well as antioxidant status of the immune system in broiler chickens (12). However, the use of steeped date seeds for improving one's health status has not been studied. Flavonoids in steeped dates increase antioxidant levels (13,14) and improve lipid profiles (15). The essential compounds in date seeds are thought to function as immunostimulants that enhance the body's immune system, though the exact mechanism of this immunostimulation is unclear. Tumor necrosis factor (TNF-α), glutathione (GSH), and gamma interferon (IFN-γ) are required for antigen binding in the inflammatory reaction. Therefore, the present study was conducted with the aim of evaluating the effect of steeped date seeds on the immune system of rats.

Materials and Methods

Preparation of steeped date seeds

Deglet nour (*Phoenix dactylifera* L.) dates were purchased from the Purwokerto Market. Date seeds were separated from the fruit, washed with tap water, drained, and then dried for one day in direct sunlight. The dried date seeds were then roasted, chopped using a blender, and filtered until only a fine powder remained.

Experimental animals

Male Wistar rats, approximately 2–3 months of age and ranging in weight from 150 to 200 g, were used in the experiment. The rats were acclimated for one week and placed in separate cages according to their experimental group. All animals had free access to food and water (ad libitum) and a 12/12-hour (light/dark) cycle was maintained. All animal experiments were performed based on standardized protocols for experimental animal studies.

Study design

This study had a laboratory experimental design with pre- and post-tests and a control group. Thirty rats were assigned to the following groups (with five rats per group): negative control (NC), positive control (PC), T1 treatment dose 1 g/kg, T3 treatment dose 3 g/kg, T5 treatment dose 5 g/kg, and healthy control (HC). Before treatment, all groups (except for the HC group) received a single dose of CCl_4. As much as 1 g of CCl_4 is diluted with 5 mL of olive oil and then given to the rats at 2 mL/kg. The steeped date-seed treatment was administered for 14 days. A total of 14 g (for T1), 42 g (for T3) and 70 g (for T5) of powdered date palm seeds were mixed with 42 mL of hot water and allowed to settle overnight. This mixture was then filtered, and 3 mL of the filtered mixture was administered to each rat every day for 14 days. The PC group received dexamethasone 0.5 mg/kg. GSH, TNF-α, and IFN-γ, were examined by enzyme-linked immunosorbent assay (ELISA) kits (BT Laboratories, Shanghai) according to the manufacturer's protocols, using an ELISA Reader machine (Labotrone, Germany).

Statistical analysis

Normally distributed data was analyzed using ANOVA followed by least significant difference (LSD) post hoc test for comparison among mean values with a significant level of 5%.

Results

This study is the first to use steeped date seeds as an anti-inflammatory agent. CCl_4, an oxidant, could induce inflammation in experimental animals. Following CCl_4 induction, GSH levels decreased (Figure 1). ANOVA revealed significant differences in GSH levels between the CCl_4-induced and HC groups ($P<0.001$). After 14 days of steeped date-seed treatment, GSH levels significantly increased ($P<0.000$). The results of the post hoc analysis showed that the T5 treatment group (5 g/kg dose) had the highest GSH level, similar to the GSH level in the HC group (Table 1); i.e., steeped date seeds could restore GSH

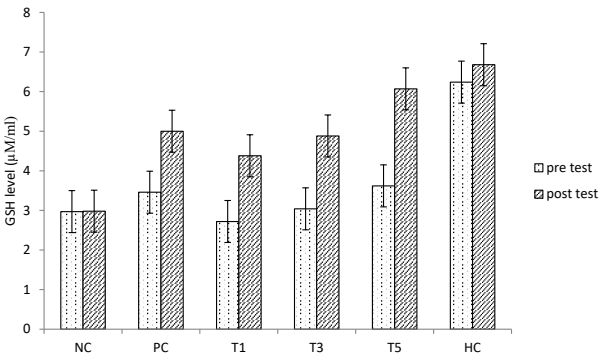

Figure 1. Glutathione (GSH) levels before and after administration of steeped date seeds. The values are the means ± standard deviation (SD). NC: negative control, PC: positive control, T1–T5: groups treated with steeped date seeds, HC: healthy control.

Table 1. Post-hoc test results of glutathione levels after date-seed treatment

Groups	NC	PC	T1	T3	T5	HC
NC	-	0.001**	0.001**	0.001**	0.001**	0.001**
PC	0.001**	-	0.026*	0.635	0.001**	0.001**
T1	0.001**	0.026*	-	0.072	0.001**	0.001**
T3	0.001**	0.635	0.072	-	0.001**	0.001**
T5	0.001**	0.001**	0.000**	0.001**	-	0.029*

NC: negative control, PC: positive control, T1–T5: groups treated with steeped date seeds, HC: healthy control.
Levels of GSH are significantly different between treatment groups and HC after administration of steeped date seeds. *$P < 0.05$, **$P < 0.01$.

in the body to almost normal levels.

Chemical mediators, such as IFN-γ, decreased in experimental animals after CCl_4-induction (Figure 2), while IFN-γ levels differed significantly between the CCl_4-induced groups and the HC group ($P < 0.001$). The levels of IFN-γ significantly increased after the administration of steeped date seeds in the treatment group ($P < 0.001$). Moreover, the LSD post-hoc test results showed that, compared to other groups, the T5 treatment group had the highest IFN-γ level (Table 2).

The TNF-α level in CCl_4-induced experimental animals was significantly higher than that of the HC group, and there was a significant difference between the levels in the induced and HC groups (Figure 3). The results showed a significant change in TNF-α level after the administration of steeped date seeds ($P < 0.001$); i.e., after the steeped date-seed treatment, TNF-α levels significantly decreased. The results of the LSD post hoc test showed that a dosage of 5 g/kg steeped date seeds was optimal and resulted in the lowest level of TNF-α (Table 3).

Discussion

Pro-inflammatory cytokines exhibit changes after exposure to free radicals. As a source of free radicals, CCl_4 is a hepatotoxic compound that causes lipid peroxidation resulting in tissue damage and inflammation. Inflammation is a dynamic process that involves pro-inflammatory cytokines, such as TNF-α, interleukin-1β (IL-1β), IL-6 and vascular endothelial growth factor, which plays a leading role in the said process (3). The need for antioxidants, such as GSH, increases during free radical exposure, resulting in a low GSH level in the body after CCl_4 exposure. GSH is synthesized from amino acids; presumably because date seeds contain various

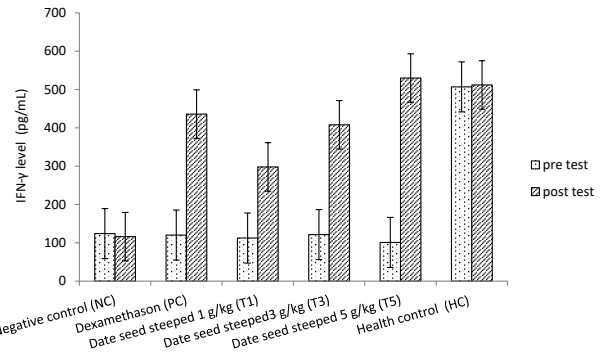

Figure 2. IFN-γ levels before and after administration of steeped date seeds. The values are the means ± standard deviation (SD). NC: negative control, PC: positive control, T1–T5: groups treated with steeped date seeds, HC: healthy control.

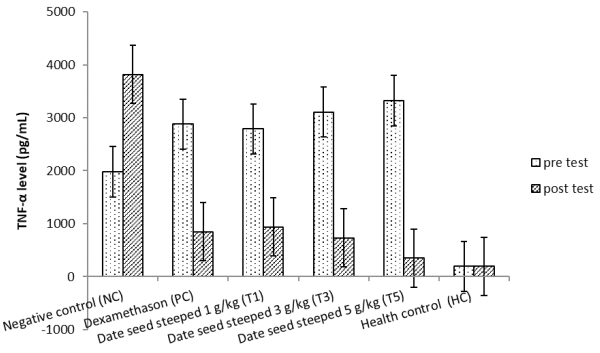

Figure 3. TNF-α levels before and after administration of steeped date seeds. The values are the means ± standard deviation (SD). NC: negative control, PC: positive control, T1–T5: groups treated with steeped date seeds, HC: healthy control.

Table 2. Post-hoc test results of IFN-γ levels after date-seed treatment

Groups	NC	PC	T1	T3	T5	HC
NC	-	0.001**	0.001**	0.001**	0.001**	0.001**
PC	0.001**	-	0.001	0.305	0.002*	0.009*
T1	0.001**	0.001**	-	0.001**	0.001**	0.001**
T3	0.001**	0.305	0.001**	-	0.001**	0.001*
T5	0.001**	0.002*	0.001**	0.001**	-	0.506
HC	0.001**	0.009*	0.001**	0.001*	0.506	-

NC: negative control, PC: positive control, T1–T5: groups treated with steeped date seeds, HC: healthy control.
Levels of IFN-γ are significantly different between treatment groups and HC after administration of steeped date seeds. *$P < 0.05$, **$P < 0.01$.

Table 3. Post-hoc test results of TNF-α levels after date-seed treatment

Groups	NC	PC	T1	T3	T5	HC
NC	-	0.001**	0.001**	0.001**	0.001**	0.001**
PC	0.001**	-	0.492	0.414	0.001*	0.001**
T1	0.001**	0.492	-	0.139	0.001**	0.001**
T3	0.001**	0.414	0.139	-	0.008*	0.001*
T5	0.001**	0.001*	0.001**	0.008*	-	0.265
HC	0.001**	0.001**	0.001**	0.001*	0.265	-

NC: negative control, PC: positive control, T1–T5: groups treated with steeped date seeds, HC: healthy control.
Comparison between groups (LSD test). *$P < 0.05$, **$P < 0.01$.

types of amino acids (4,5), administering steeped date seeds increases GSH levels.

Reduced GSH plays an important role in various biological processes, such as oxidation reduction, transport, protein synthesis, catabolism, and metabolism. High levels of free radicals in the body require a large number of antioxidants to combat them. The results of this study are consistent with previous research, demonstrating that steeped date seeds can improve GSH peroxidase in premenopausal women (14).

The initial immune response to inflammation induced by CCl_4 is characterized by a decrease in IFN-γ production. Decreased IFN-γ depresses macrophage function, allowing the inflammatory process to continue. Several studies have shown that date seeds can significantly stimulate IFN-γ and cell mRNA expression and improve the Th1 immune response (16). These effects have been related to the polyphenol and polysaccharide content in date seeds. Administering date palm extract for seven days can increase antibody titers and plaque-forming cells. The phenolic and flavonoid components of date seeds may also act as anti-inflammatory inhibitors of cyclooxygenase (COX2) enzymes. Animal studies have shown that dates have potential protective effects via modulation of cytokine expression (17). Other findings suggest that dates also play a role in reducing leg swelling and plasma fibrinogen (18).

IFN-γ has immuno-regulator activity and plays a role in the differentiation of B cells and macrophage activation. IFN-γ has also an antiviral activity. Increased immune activity leads to higher IFN-γ production and increased antigen-presenting cell function via class II molecular induction. IFN-γ is produced by activated T cells as well as natural killer (NK) cells. Treatment with steeped date seeds stimulates T cells, increasing the production of IFN-γ, which suggests favorable cellular immune responses. The results of this study indicate that steeped date seeds can induce macrophages to produce IFN-γ.

The dominant components in date seeds are polysaccharides, such as pectin, β-glucan and polyphenols. Pectin plays an important role in immuno-modulatory effects, including protecting against bacterial streptococcus infections and upregulating IL-1β and IFN-γ. β-glucan has been found to protect against bacterial and protozoan infections in experimental animals (16). Palm water can also act to increase CD4 + T cells in mice. CD4+ is a marker of the surface antigen of T lymphocyte cells; thus, increasing CD4 + T cells leads to an increase in lymphocyte cells. CD4 + stimulates Th2 activation, while Th2, in turn, stimulates B lymphocyte cells to produce antibodies in the form of immunoglobulin. Cytotoxic T (Tc) cells activate IFN-γ, and IFN-γ activates macrophages, increasing respiratory burs and resulting in increased bactericidal activity. CD8+ T cells activate Tc and serve as a Tc cell surface marker.

Lymphocytes proliferate and differentiate into T cells and B cells, both of which play a role in specific immune responses. Macrophages perform pathogenic phagocytosis and play a role in nonspecific immune responses. Chemical mediators (cytokines) help control immune responses, such as the IFN-γ produced by T cells, which activate macrophages and NK cells. IFN-γ and other cytokines play an important role in the activation of cellular immunity. Moreover, IFN-γ increases human leukocyte antigen (HLA) expression on the surface of virus-infected cells, allowing HLA with a viral antigen on the cell surface to be recognized by Tc cells, leading to cell lysis. IFN-γ can also promote attacks against viral, bacterial and parasitic infections, as well as inhibit cell division and cell differentiation. The results of this study are in line with other studies, demonstrating that steeped date seeds can improve IFN-γ levels (12).

Date seeds can boost the immune system because they contain many active substances. For example, date seeds contain various phenolic compounds, such as ferulic acid, p-coumaric, quercetin, isorhamnetin heterosides, p-hydroxybenzoic and caffeoylshikimic acids, catechin and epicatechin flavanols (19,20), tannins, and carbohydrates. Date seeds also contain certain antioxidants and antioxidant enzymes, such as polyphenol oxidase, antioxidant vitamins, alkaloids, steroids, flavonoids, and oleic acid (21,22). These components are excellent anti-inflammatories. Moreover, phenols have antiseptic properties. The protein content in palm kernels is quite high, accounting for two-thirds of the flesh (23). Date seeds contain abundant amounts of

fiber (73.1 g/100 g), phenolics (3942 mg/100 g), fat (9.0 g/100 g), protein (5.1 g/100 g), and antioxidants (24). Moreover, date seeds contain many phenolic compounds and natural dietary fiber sources, as well as anti-oxidants (25), which contribute their anti-inflammatory activities to the date palm. Phenolic compounds are believed to inhibit inflammation through mechanisms that inhibit leukocyte migration and also reduce serum lysozyme levels, nitric oxide (NO), prostaglandin E2 (PGE2) and malondialdehyde (26).

Compared to raw date seeds, roasted date seeds have a higher flavonoid content. Roasted date seed flavonoids contain rutin (2.2 mg/gm), gallic acid (0.1 mg/gm), and quercetin (0.52 mg/gm), while raw date seeds contain rutin (0.4 mg/gm), gallic acid (0.1 mg/gm), and quercetin (0.9 mg/gm) (27). Flavonoids can inhibit lipoxygenase activity, PGE2 synthesis, COX2, NO production, and TNF-α-mediated monocyte adhesion, which suppresses pro-inflammatory gene expression (26). Date seeds also contain high levels of vitamins E and C. The anti-inflammatory effect of Phoenix dactylifera is related to its unsaturated fatty acids, antioxidant compounds (vitamins E and C) and phenolic compounds (28). Date-seed extract can decrease inflammation in pancreatic beta cells, increase the production of insulin (29) and decrease the proliferation of pancreatic cancer cells, superoxide-dependent iron release and DNA damage (30).

Conclusion

CCl_4 induction decreases GSH and IFN-γ while increases the TNF-α. Administration of steeped date seeds increases GSH and IFN-γ, but decreases TNF-α. The findings of this study are important regarding the potential for the use of steeped date seeds as anti-inflammatory compounds. This research will help future researchers to explain the mechanism of action behind the anti-inflammatory effect of the steeped date seed.

Acknowledgments

The researchers wish to express their deepest gratitude to the Institute for Research and Community Service, University of Jenderal Soedirman through the Institutional Research Fund (contract number: 4869/UN23.14/PN/2017).

Authors' contributions

SS and AT designed the study and protocol. SS and AP carried out data collection. SS, AT and AP analyzed the results of the study. SS, AP, and FE interpreted the study results. SS, AT, AP and FE drafted the manuscript. All authors contributed to data analysis, drafting and critically revising the paper. All authors have read the final version and have agreed to publish it.

Conflict of interests

None declared.

Ethical considerations

This study was conducted with approval and ethical clearance from the Medical Research Ethics Commission (contract number 331/IV/HREC/2017) of the Faculty of Medicine, Universitas Sebelas Maret, Solo—Indonesia.

Funding/Support

This research was supported by the Institutional Research Grant (contract number: 4869/UN23.14/PN/2017) from the Institute for Research and Community Service, University of Jenderal Soedirman.

References

1. Lovric J, Mesic M, Macan M, Koprivanac M, Kelava M, Bradamante V. Measurement of malondialdehyde (MDA) level in rat plasma after simvastatin treatment using two different analytical methods. Period Biol. 2008;110(1):63-8.
2. Bisht S, Sisodia SS. Diabetes, dyslipidemia, antioxidant and status of oxidative stress. Int J Res Ayurveda Pharm. 2010;1(1):33-42.
3. Mehta SK, Gowder SJT. Members of antioxidant machinery and their functions. In: Basic Principles and Clinical Significance of Oxidative Stress. InTech; 2015.
4. Sheikh BY, Zihad SM, Sifat N, Uddin SJ, Shilpi JA, Hamdi OA, et al. Comparative study of neuropharmacological, analgesic properties and phenolic profile of Ajwah, Safawy and Sukkari cultivars of date palm (*Phoenix dactylifera*). Orient Pharm Exp Med. 2016;16(3):175-83. doi: 10.1007/s13596-016-0239-5.
5. Abdelaziz DH, Ali SA. The protective effect of *Phoenix dactylifera* L. seeds against CCl4-induced hepatotoxicity in rats. J Ethnopharmacol. 2014;155(1):736-43. doi: 10.1016/j.jep.2014.06.026.
6. Eddine KH, Zerizer S, Kabouche Z. Immunostimulatory activity of *Phoenix dactilifera*. Int J Pharm Pharm Sci. 2014;6(3):73-6.
7. Yasin BR, El-Fawal HA, Mousa SA. Date (*Phoenix dactylifera*) Polyphenolics and Other Bioactive Compounds: A Traditional Islamic Remedy's Potential in Prevention of Cell Damage, Cancer Therapeutics and Beyond. Int J Mol Sci. 2015;16(12):30075-90. doi: 10.3390/ijms161226210.
8. Zhang CR, Aldosari SA, Vidyasagar PS, Nair KM, Nair MG. Antioxidant and anti-inflammatory assays confirm bioactive compounds in Ajwa date fruit. J Agric Food Chem. 2013;61(24):5834-40. doi: 10.1021/jf401371v.
9. Takaeidi MR, Jahangiri A, Khodayar MJ, Siahpoosh A, Yaghooti H, Rezaei S, et al. The effect of date seed (*Phoenix dactylifera*) extract on paraoxonase and arylesterase activities in hypercholesterolemic rats. Jundishapur J Nat Pharm Prod. 2014;9(1):30-4.
10. Puri A, Sahai R, Singh KL, Saxena RP, Tandon JS, Saxena KC. Immunostimulant activity of dry fruits and plant materials used in Indian traditional medical system for mothers after child birth and invalids. J Ethnopharmacol. 2000;71(1-2):89-92.
11. Habib HM, Platat C, Meudec E, Cheynier V, Ibrahim WH. Polyphenolic compounds in date fruit seed (*Phoenix dactylifera*): characterisation and quantification by using UPLC-DAD-ESI-MS. J Sci Food Agric. 2014;94(6):1084-9.

doi: 10.1002/jsfa.6387.
12. El-Far AH, Ahmed HA, Shaheen HM. Dietary supplementation of *Phoenix dactylifera* seeds enhances performance, immune response, and antioxidant status in broilers. Oxid Med Cell Longev. 2016;2016:5454963. doi: 10.1155/2016/5454963.
13. Saryono S, Sumeru A, Proverawati A, Efendi F. Decreasing Carbon Tetrachloride Toxicity using Date-seed (*Phoenix dactylifera* L.) Steeping in Rats. Toxicol Environ Health Sci. 2018;10(2):139-45. doi: 10.1007/s13530-018-0357-1.
14. Saryono S, Rahmawati E, Proverawati A, Hisni D. Effect of antioxidant status and oxidative stress products in pre-menopausal women after treatment with date seed powder (*Phoenix dactylifera* L.): A study on women in Indonesia. Pak J Nutr. 2017;16(6):477-81. doi: 10.3923/pjn.2017.477.481.
15. Saryono S, Eliyan J, Herdiati D, Khikmatullah AA, Silvana CP, Adi HP. Anti-atherogenic properties of Deglet Noor Date seeds (*Phoenix dactylifera*) Methanol extract on Diet-Induced Hypercholesterolemic Rats. IOP Conf Ser Mater Sci Eng. 2017;172:012046. doi: 10.1088/1757-899x/172/1/012046.
16. Karasawa K, Uzuhashi Y, Hirota M, Otani H. A matured fruit extract of date palm tree (*Phoenix dactylifera* L.) stimulates the cellular immune system in mice. J Agric Food Chem. 2011;59(20):11287-93. doi: 10.1021/jf2029225.
17. Elberry AA, Mufti ST, Al-Maghrabi JA, Abdel-Sattar EA, Ashour OM, Ghareib SA, et al. Anti-inflammatory and antiproliferative activities of date palm pollen (*Phoenix dactylifera*) on experimentally-induced atypical prostatic hyperplasia in rats. J Inflamm (Lond). 2011;8(1):40. doi: 10.1186/1476-9255-8-40.
18. Mohamed DA, Al-Okbi SY. In vivo evaluation of antioxidant and anti-inflammatory activity of different extracts of date fruits in adjuvant arthritis. Pol J Food Nutr Sci. 2004;13(4):397-402.
19. Bouhlali ET, Alem C, Ennassir J, Benlyas M, Mbark AN, Filali-Zegzouti Y. Phytochemical compositions and antioxidant capacity of three date (*Phoenix dactylifera* L.) seeds varieties grown in the South East Morocco. J Saudi Soc Agric Sci. 2017;16(4):350-7. doi: 10.1016/j.jssas.2015.11.002.
20. Platat C, Habib HM, Al Maqbali FD, Jaber NN, Ibrahim WH. Identification of date seeds varieties patterns to optimize nutritional benefits of date seeds. J Nutr Food Sci. 2014;S8:1-8. doi: 10.4172/2155-9600.S8-008.
21. Biglar M, Khanavi M, Hajimahmoodi M, Hassani S, Moghaddam G, Sadeghi N, et al. Tocopherol content and Fatty Acid profile of different Iranian date seed oils. Iran J Pharm Res. 2012;11(3):873-8.
22. Hamada JS, Hashim IB, Sharif FA. Preliminary analysis and potential uses of date pits in foods. Food Chem. 2002;76(2):135-7.
23. Bhat RS, Al-Daihan S. Antibacterial properties of different cultivars of *Phoenix dactylifera* L. and their corresponding protein content. Ann Biol Res. 2012;3(10):4751-7.
24. Baliga MS, Baliga BRV, Kandathil SM, Bhat HP, Vayalil PK. A review of the chemistry and pharmacology of the date fruits (*Phoenix dactylifera* L.). Food Res Int. 2011;44(7):1812-22. doi:10.1016/j.foodres.2010.07.004.
25. Al-Farsi MA, Lee CY. Nutritional and functional properties of dates: a review. Crit Rev Food Sci Nutr. 2008;48(10):877-87. doi: 10.1080/10408390701724264.
26. Beg S, Swain S, Hasan H, Barkat MA, Hussain MS. Systematic review of herbals as potential anti-inflammatory agents: Recent advances, current clinical status and future perspectives. Pharmacogn Rev. 2011;5(10):120-37. doi: 10.4103/0973-7847.91102.
27. Paranthaman R, Praveen Kumar P, Kumaravel S. HPLC and HPTLC determination of caffeine in raw and roasted date seeds (*Phoenix Dactylifera* L.). Open Access Scientific Report. 2012;1(4):1-4. doi: 10.4172/scientificreports.249.
28. Rahmani AH, Aly SM, Ali H, Babiker AY, Srikar S, Khan AA. Therapeutic effects of date fruits (*Phoenix dactylifera*) in the prevention of diseases via modulation of anti-inflammatory, anti-oxidant and anti-tumour activity. Int J Clin Exp Med. 2014;7(3):483-91.
29. El Fouhil AF, Ahmed AM, Atteya M, Mohamed RA, Moustafa AS, Darwish HH. An extract from date seeds stimulates endogenous insulin secretion in streptozotocin-induced type I diabetic rats. Funct Food Health Dis. 2013;3(11):441-6. doi: 10.31989/ffhd.v3i11.33.
30. Habib HM, Platat C, Al Maqbali FD, Ibrahim WH. Date seed (*Phoenix dactylifera*) extract reduces the proliferation of pancreatic cancer cells, DNA damage and superoxide-dependent iron release from ferritin in vitro (829.20). FASEB J. 2014;28(1 Suppl):820-9.