Sains Malaysiana 47(11)(2018): 2741–2755
http://dx.doi.org/10.17576/jsm-2018-4711-17

Ficus carica and Bone Health: A Systematic Review

(*Ficus carica* dan Kesihatan Tulang: Suatu Kajian Sistematik)

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ABSTRACT

Ficus carica, *a native plant to the Middle East and Western Asia, is of high value in folk medicine. The therapeutic potential of* Ficus carica *has led to the extensive studies in recent years, focusing on evaluating and validating its pharmacological effect. The present systematic review summarizes the effectiveness of* Ficus carica *on promoting bone health focusing on osteoporosis and rheumatoid arthritis via mineral contents and RANKL pathway. The search was done with Medline via Ebscohost, Scopus and Google Scholar databases to obtain relevant articles published between 1946 and December 2016. The main inclusion criteria were research articles published in English that reported effect of* Ficus carica *on bone health. The literature search returned 716 potentially relevant articles, whereby 5 met the inclusion criteria. This systematic review concludes* Ficus carica *plays an important role in the promotion of bone health and can be a potential pharmaceutical product in the future.*

Keywords: Bone; Ficus carica*; osteoporosis; RANKL pathway; rheumatoid arthritis*

ABSTRAK

Ficus carica *ialah tumbuhan asli di Timur Tengah dan Asia Barat mempunyai kepentingan dalam perubatan tradisi. Potensi terapeutik* Ficus carica *telah membawa kepada kajian mendalam tertumpu kepada pengesahan kesan farmakologinya. Kajian sistematik ini mendalami keberkesanan* Ficus carica *dalam membantu kesihatan tulang yang memfokus kepada penyakit osteoporosis dan 'reumatoid artritis' melalui kandungan mineralnya dan laluan RANKL. Carian makalah telah dibuat menggunakan pangkalan data Medline melalui Ebscohost, Scopus dan Google Scholar untuk mendapatkan makalah berkaitan yang diterbitkan antara 1946 dan December 2016. Kriteria rangkuman utama untuk pemilihan makalah adalah penerbitan dalam Bahasa Inggeris yang melaporkan kesan* Ficus carica *kepada kesihatan tulang. Carian makalah menghasilkan 716 makalah yang berpotensi dengan 5 makalah menepati kriteria rangkuman. Kesimpulan kajian sistematik ini membuktikan bahawa* Ficus carica *memainkan peranan penting dalam membantu meningkatkan kesihatan tulang dan boleh dijadikan sebagai produk farmaseutik yang berpotensi pada masa hadapan.*

Kata kunci: Ficus carica*; laluan RANKL; osteoporosis; reumatoid artritis; tulang*

INTRODUCTION

Ficus carica or commonly known as fig is a flowering plant belongs to the Moraceae family. It is one of the largest genera of angiosperms with more than 800 species of trees, epiphytes, and shrubs identified in the tropical and sub-tropical regions worldwide (Vinson 1999). It is known to be one of the earliest fruits cultivated in history (Singh et al. 2011). *Ficus carica* has been extensively studied for medicinal uses, which justifies its potential therapeutic value (Khairuddin et al. 2017; Mawa et al. 2013; Moniruzzaman et al. 2017). A report done by Gilani et al. (2008) showed that the fruit, root and leaves of *Ficus carica* are used in complementary medicine in different disorders such as spasm, respiratory disorders, inflammatory, gastro-intestinal disorders and cardiovascular disorders.

The therapeutic potential of *Ficus carica* has led to the extensive studies in recent years, focusing on evaluating and validating its pharmacological effect. *Ficus carica* has been reported to possess antioxidant activity (Feng & Ma 2010; Prabavathy & Nachiyar 2011), anti-angiogenic activity, anticancer activity (Rubnov et al. 2001), antibacterial activity (Aref et al. 2010; Jeong et al. 2009; Mavlonov et al. 2008), cytotoxicity activity (Khodarahmi et al. 2011), anticonstipation activity (Lee et al. 2012), hepatoprotective activity (Gond et al. 2008; Mohan et al. 2007), antihelmintic activity (Amol et al. 2010; Chandrashekhar et al. 2008), anti-inflammatory activity (Patil et al. 2011), antimutagenic activity (Agabeĭli et al. 2005), antipyretic activity (Patil et al. 2010), antispasmodic activity (Gilani et al. 2008), antiplatelet activity (Gilani et al. 2008), hypoglycemic activity (El- Shobaki et al. 2010; Perez et al. 1996), hypolipidimic activity (Asadi et al. 2006), antiviral activity (Lazreg Aref et al. 2011) and immunostimulant activity (Patil et al. 2010).

Fruits such as olive (Chin & Ima-Nirwana 2016) and pomegranate (Shuid & Mohamed 2013) as an alternative

dietary supplementation for osteoporosis have been suggested for individual who preferred vegan diet or those who have medical condition that prevents them from consuming dairy or meat product such as milk allergy.

Mineral content of fig has been reported to closely resemble that of human milk, with iron being the most important. The iron content in *Ficus carica* is also said to be 50% as much as that of beef liver (Lydia 2009). In plant eating bird, *Ficus carica* has been reported to be the choice for dietary source of calcium by O'Brien et al. (1998). Together, their findings suggested the potential of *Ficus carica* as an alternative dietary supplement for prevention of osteoporosis.

Bone problem such as bone loss, osteoporosis and rheumatoid arthritis are a global health problem. Aging can reduce bone mineral density (BMD), eventually leading to osteoporosis. Dairy products with high level of calcium are recommended to be consumed in order to promote and maintain BMD level, especially in the elderly. Apart from being nutritional source of minerals, *Ficus carica* has also been reported to modulate bone remodelling (Choi et al. 2011; Park et al. 2009). The mechanism of bone remodeling is composed of a balance between bone resorption phase regulated by osteoclast and bone formation phase regulated by osteoblast (Liu et al. 2010; Raggatt et al. 2010). The imbalance of bone remodeling process caused by an excessive differentiation of osteoclast cells has been previously reported, that can lead to bone lytic diseases, such as osteoporosis and rheumatoid arthritis (Park et al. 2008).

The activation of osteoclasts is known to be regulated by two cytokines; receptor activator of nuclear factor-κβ ligand (RANKL) and macrophage colony-stimulation factor (M-CSF). The binding of RANKL to its receptor RANK on the surface of osteoclast, leads to the activation of TNF receptor-associated factor 6 (TRAF6), which is linked to nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) via mitogen-activated protein kinases (MAPKs). RANKL and M-CSF are proteins secreted by osteoblasts and is important for the formation of osteoclast and regulation of its activity (Boyce & Xing 2008).

Studies led by Choi et al. (2011) and Park et al. (2009), respectively, have showed that *Ficus carica* can act as a potent inhibitor of osteoclastogenesis in receptor activator of nuclear factor kappa-B ligand (RANKL) pathway and regulates expression of osteoblast specific genes such as bone morphogenetic protein 2 (BMP-2), osteoprotegerin (OPG) and osteocalcin (OCN). The importance of RANKL signaling mechanism in bone remodelling is well established (Liu et al. 2010; Raggatt et al. 2010). Both studies suggested that *Ficus carica* may potentially provide a novel therapy for bone disorder such as osteoporosis (Choi et al. 2011; Park et al. 2009).

In this review, studies reporting the beneficial effect of *Ficus carica* on bone health via its high mineral contents and its potential to inhibit osteoclastogenesis will be discussed.

Methods

Search strategy

A systematic review of the literature was conducted to identify relevant studies reporting the effects of *Ficus carica* on bone health. Two databases were searched in regard to this, Medline via Ebscohost and Scopus (both published between 1946 and December 2016) and Google Scholar (no limitation in search). The search strategy involved a combination of the following sets of key words; *Ficus carica* AND osteo* OR rheum* OR bone*.

Inclusion and exclusion criteria

The results were limited only to the studies published in English language due to limited resources for translation services. Primary literature with research focus on *Ficus carica* effects on bone health was included. Review articles, news, letter, editorials or case studies were excluded from the review. Study not related to *Ficus carica* effects on bone health were removed.

Data extraction and management

Articles were screened prior to their inclusion in this review. Titles and abstracts were screened first to ensure inclusion and exclusion criteria were adhered. In the final phase, the remaining papers were read thoroughly and the data extracted. The following data were recorded from the studies: the types of study; aims of study; subject or sample; methods; result; and remarks or conclusion. All the data extraction and management were re-evaluated by two independent reviewers to validate the data integrity.

Results

Literature search

The literature identified several relevant articles. All the articles were assessed for inclusion or exclusion based on the title and abstract. The search yielded 716 articles, of which 5 articles met the inclusion criteria. All data were extracted directly from the articles. A flow chart of the selection and paper process including reasons for exclusion is shown in Figure 1. Further details on each study regarding methodological and outcome aspects were summarized in Table 1.

Evaluation of major mineral contents of *Ficus carica* crucial for bone health

Three studies were included in this review to evaluate the mineral contents of *Ficus carica* that is crucial for bone health (Khan et al. 2011; Sadia et al. 2014; Soni et al. 2014). Khan et al. (2011) was the earliest to conduct physicochemical profiling of *Ficus carica* (Sadia et al. 2014). Their findings showed that their fig samples contain high amount of potassium (K) at 382.4-611.5 mg/100 g. This is followed by magnesium (Mg) at 110.50-202.40

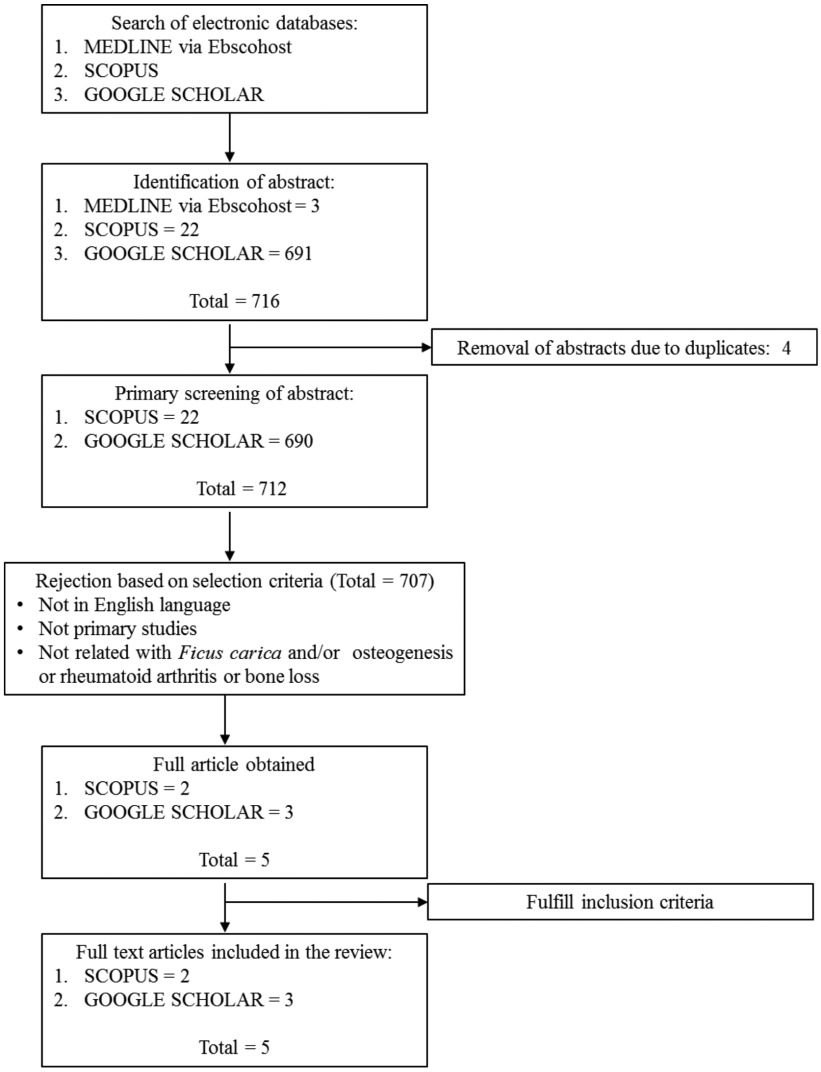

FIGURE 1. Flow chart of the search strategy, study selection and data management procedure

mg/100 g, Calcium (Ca) 78.72-132.80 mg/100 g and phosphorous (P) at 31.91-76.96 mg/100 g (Khan et al. 2011). They suggested that the minerals found in *Ficus carica* fruit are essential for bone growth and maintenance.

Sadia et al. (2014) has led a study to evaluate the physicochemical characteristics of a few species of underutilized figs and mulberries. It showed that the dried figs extract contains higher concentration of trace elements such as potassium (K), magnesium (Mg), calcium (Ca), phosphorus (P) and Iron (Fe) [31]. Accordingly, *Ficus carica* was reported to have the highest level of Ca ((10.94 ± 2.75) mg/g dry weight) among all the fruits tested (Sadia et al. 2014).

This is in agreement to another study led by Soni et al. (2014) that reported dried fig to be a very good source of minerals like Strontium (Sr), Ca, Mg, P and Fe (Soni et al. 2014). They found that Strontium has the highest amount in fig (saturated) while the level of Ca, Mg, P and Fe to be relatively high at 1545.46, 679.04, 365.75 and 29.49 ppm, respectively. Strontium and calcium in dried fig has been found to contribute towards good bone health (Soni et al. 2014). In terms of nutritional profile, fig have carbohydrates as a major component (73.50%), high energy value (317.78 kcal), very low amount of fat (0.56%), moderate amount of protein (4.67%), dietary fiber content (3.68%), found to contain moisture (16.63%) and high ash content (4.65%) (Soni et al. 2014).

EFFECT OF *FICUS CARICA* ON MOLECULAR MECHANISM OF BONE HEALTH

Studies led by Choi et al. (2011) and Park et al. (2009) have successfully linked *Ficus carica* to the molecular mechanism of bone formation via the RANKL signaling. In the first study, Choi et al. (2011) tested the effect of four types of long chain polyunsaturated fatty acids (PUFAs) from *Ficus carica*, namely E-DHA, DHA, EDA (cis-11, 14-eicosadienoic acid) and EPA on the osteogenesis parameters of RAW264.7 murine macrophages and preosteoblastic MC3T3-E1 cells.

In terms of RAW 264.7 cells, E-DHA was found to be a much more potent inhibitor of RANKL-induced osteoclastogenesis (Choi et al. 2011). TRAP staining showed

TABLE 1. Characteristic summaries of studies included in the review

Evaluation of mineral contents of *Ficus carica* for osteoporosis and rheumatoid arthritis						
Reference	Types of study	Aim of study	Subject/Sample	Methods (Parameters)	Results	Remarks/Conclusion
Sadia et al. (2014)(31).	Identification study on physicochemical characterization of species of Figs and Mulberries	To identify alternative bio-nutritional sources, some underutilized species of Figs and Mulberries	Fresh fully ripened fruits: 1. Figs (*Ficus carica* L., *F. palmata* Forssk., *F. racemosa* L.) 2. Mulberries (*Morus alba* L., *M. nigra* L., *M. laevigata* Wall.)	- Sampling were performed by oven-dried at 68°C for 24 h and ground into fine powder for nutritional analysis - Proximate analysis: To determine the proximate composition of fruit samples such as: 1. Moisture value (g·100 g^{-1} fresh weight) 2. Ash value (g·100 g^{-1} dry weight) 3. Fats value: (g·100 g^{-1} dry weight) 4. Fiber value: (g·100 g^{-1} dry weight) 5. Protein value: (g·100 g^{-1} dry weight) 6. Carbohydrates value: (g·100 g^{-1} dry weight) 7. Energy value: (kcal·100 g^{-1} dry weight) - Mineral assessment: To determine inorganic macro-elements and micro-elements Inorganic macro-elements: 1. Nitrogen (N) 2. Phosporus (P) 3. Sodium (Na) 4. Potassium (K) 5. Calcium (Ca) 6. Magnesium (Mg) Inorganic micro-elements: 7. Iron (Fe) 8. Manganese (Mn) 9. Zinc (Zn) 10. Lead (Pb) 11. Nikerl (Ni) 12. Cobalt (Co) 13. Strontium (Sr) 14. Chromium (Cr)	Proximate analysis: 1. Moisture value: mulberries contain higher moisture content [(80.37 ± 0.14) g·100 g^{-1} fresh weight in *M. laviegata*) than figs [(17.82 ± 0.21) g·100 g^{-1} fresh weight in *F. carica*] 2. Ash value: higher in *M. alba* and *F. carica* (4.55 ± 0.09 and 4.50 ± 0.41 g·100 g^{-1} dry weight) 3. Fats value: highest quantity in *F. glomerata* [(2.71 ± 0.03) g·100 g^{-1} dry weight] 4. Fiber value: high crude fiber content [(17.81 ± 0.03) g·100 g^{-1} dry weight] in *F. palmata* 5. Protein value: higher in *M. alba* [(13.50 ± 0.28) g·100 g^{-1} dry weight] 6. Carbohydrates value: higher in *M. nigra* (75.58 ± 0.54) g·100 g^{-1} dry weight 7. Energy value: highest value in *M. laviegata* (367.74 ± 0.35 kcal·100 g^{-1} dry weight) - Mineral assessment: Inorganic macro-elements: 1. N: highest in *M. laviegata* (0.24 ± 0.07 mg·g^{-1} dry weight) 2. P: highest in *F. glomerata* (1.5 ± 0.93 mg·g^{-1} dry weight) 3. Na: higher in *F. palmata* (1.92 ± 0.11 mg·g^{-1} dry weight) 4. K: higher in *F. glomerata* (17.21 ± 0.03 mg·g^{-1} dry weight) 5. Ca: highest in *F. carica* (10.94 ± 2.75 mg·g^{-1} dry weight) 6. Mg: highest in *F. palmata* (6.92 ± 0.37 mg·g^{-1} dry weight) Inorganic micro-elements: 7. Fe: highest in *M. laviegata* (1.43 ± 0.42 mg·g^{-1} dry weight) 8. Mn: highest in *F. glomerata* (0.95 ± 0.05 mg·g^{-1} dry weight)	Highest calcium was found in *Ficus carica*. Calcium is a major component of bone and assisted in tooth development

Continues TABLE 1.

Evaluation of mineral contents of *Ficus carica* for osteoporosis and rheumatoid arthritis						
Reference	Types of study	Aim of study	Subject/Sample	Methods (Parameters)	Results	Remarks/Conclusion
					9. Zn: highest in *F. palmata* (0.52 ± 0.14 mg·g^{-1} dry weight) 10. Pb: highest in *M. alba* (1.00 ± 0.11 mg·g^{-1} dry weight) 11. Ni: highest in *M. nigra* (0.16 ± 0.10 mg·g^{-1} dry weight) 12. Co: highest in *F. carica* (0.08 ± 0.05 mg·g^{-1} dry weight) 13. Sr: highest in *F. glomerata* (0.06 ± 0.02 mg·g^{-1} dry weight) 14. Cr: highest in *F. glomerata* (2.91 ± 0.14 mg·g^{-1} dry weight)	
Soni et al. (2014)(32).	Identification study on phytochemical antioxidant and antibacterial activity of dried fruit of *Ficus carica*	To investigate the nutritional, phytochemical, antioxidant and antibacterial activity of dried fruit of *Ficus carica*	Dried fig fruit	- Sampling: Dried fig fruit was dried at 40°C in an oven and coarsely powdered using a mixer grinder - Extract preparation: Four solvents was used for extraction; acetone, dichloromethane, ethyl acetate and methanol. 50 g of powdered sample of dried fruit of fig was weighed. Soaked in 35 mL of each of 4 solvents and incubated at 40°C with 140 rpm for 48 h. The mixture was filtered and the extract obtained after double extraction with solvents was dark yellow brown in color and extract yield of dried fig fruit powder was calculated to be 11.47% - Nutritional profiling: To determine energy value, total carbohydrate, fat, protein, dieatry fiber, moisture and ash content - Mineral content analysis was performed using an inductively coupled plasma optical emission spectrometry (ICP-OES): To determine minerals content of dried figs - Phytochemical analysis: To screen a total phenolics, total flavonoids, alkaloids and saponins of dried figs	- Nutritional profiling: Dried fruit of fig has carbohydrates as a major component (73.50%), high energy value (317.78 kcal), very low amount of fat (0.56%), moderate amount of protein (4.67%), dietary fiber content (3.68%), found to contain moisture (16.63%) and high ash content (4.65%) - Mineral content profiling: Dried fig has high amount of Strontium (Sr). Calcium, Magnesium, Phosphorous and Iron were found to be a very good of mineral sources in dried fig (1545.46, 679.04, 365.75, 29.49 in ppm) - Phytochemical analysis: Total phenolics and flavonoids content of fig extract were found to be in moderate amounts (10.90 µg GAE/mg sample and 2.75 µg CE/ mg sample). Crude alkaloid was found high amount and saponins very low amount (9.6% and 0.59% in g/100 g DM) - Secondary metabolites analysis: A total of 68 compounds were identified in fig. The major compounds were Beta-Amyrin, Stigmasterol, Campesterol, gamma sitosterol, oleic acid, Isoamyl laurate, α-tocopherols, ϒ tocopherols, β-amyrins, Oleic acid, Isoamyl laurate, Vitamin E The major components detected in volatile oil of the fruits were furfural (10.55%), 5-methyl-2-furaldehyde (10.1%), and benzeneacetaldehyde (6.59%)	Strontium and calcium in dried fig has been found to contribute towards good bone health *Ficus carica* may be utilized as nutraceutical food with high nutrition and therapeutic benefits

Continues TABLE 1.

Evaluation of mineral contents of *Ficus carica* for osteoporosis and rheumatoid arthritis						
Reference	Types of study	Aim of study	Subject/Sample	Methods (Parameters)	Results	Remarks/Conclusion
				- Secondary metabolites analysis was performed using mass spectrometric and automatic RTL screener software to determine secondary metabolites of dried figs. - 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) or ABTS radical scavenging assay and Ferric reducing antioxidant power (FRAP) were performed to determine antioxidant activity of dried figs - Antimicrobial susceptibility assay to determine antibacterial activity of dried figs	- Antioxidant activity (IC_{50} value) and FRAP activity were found very good in fig (19.8 mg/mL and 60.48) - Antimicrobial susceptibility test found 100 mg/mL of dried fig extract inhibited *Bacillus subtilis* and *Proteus mirabilis*	
Khan et al. (2011)(33)	Identification study on phytochemical of *Ficus carica*	To assess the nutritional characteristics of the *Ficus carica* fruit in Pakistan by studying some of its physico-chemical properties	Fully ripened fresh *Ficus carica*	- Sampling were taken 500 g of each group: 1. S1-S4 were cultivated in Baluchistan province 2. S5 and S6 were grown and utilized in Sindh 3. S7 was cultivated in Punjab - Proximate composition analysis was performed to determine the water content, volatile matter and ash content of the fruit 1. The estimation of water content were held at 105°C in nitrogen atmosphere to remove water until a constant weight was obtained and calculated by taking into account the difference between initial mass and the constant mass at 105°C 2. The volatile measurement was performed by increasing the furnace temperature with a ramp rate of 16°C min^{-1}. The amount of volatile matter was estimated after a constant heating at 950°C for 7 min in a nitrogen environment to ensure complete devolatilisation	- Proximate composition analysis: 1. The water content in the samples was in the range of 12.89-17.50 g/100 g (average = 14.86 g/100 g) 2. The amount of volatiles in the samples was in the range of 79.81-82.25 g/100 g (average: 80.77 g/100 g) 3. The amount of ash in the samples was in the range of 1.39-2.31 g/100 g (average: 1.95 g/100 g) - Calorific and acidity values of all samples were ranged from 337.60-364.70 kcal/100 g (average: 350.34 kcal/100 g) and ranged from 0.35-0.69 g/10g in g/100 g of oxalic acid - Mineral nutrient content of all samples showed that 1. Ca: 78.72-132.80 mg/100 g 2. Mg: 110.50-202.40 mg/100 g 3. K: major mineral element (382.4-611.5 mg/100 g). 4. Na: 5.58-17.84 mg/100 g 5. Fe: 5.69-10.09 mg/100 g 6. Zn: 0.32-0.62 mg/100 g 7. Cu: 0.25-0.42 mg/100 g 8. Co: 0.1 mg/L 9. Ni: 0.1 mg/L 10. P: 31.91-76.96 mg/100 g	- Potassium was found as major mineral in *Ficus carica fruit* and good for bone - Calcium was found as highest mineral in *Ficus carica* fruit which is an essential for bone health

Continues TABLE 1.

Reference	Types of study	Aim of study	Subject/Sample	Methods (Parameters)	Results	Remarks/Conclusion
				3. The ash content was determined by increasing the furnace temperature to a constant temperature of 750°C with the same ramp rate in an air flux of 20 mL min^{-1} - Calorific value analysis was performed to determine calorific values of the samples - Mineral nutrient content by spectrophotometric analysis was performed to establish the minerals of interest in fruit such as: 1. Calcium (Ca) 2. Magnesium (Mg) 3. Potassium (K) 4. Sodium (Na) 5. Iron (Fe) 6. Zinc (Zn) 7. Copper (Cu) 8. Cobalt (Co) 9. Nickel (Ni) 10. Phosphorus (P)		
Effect of *Ficus carica* on molecular mechanism of osteoporosis and rheumatoid arthritis						
Choi et al. (2011)(26)	*In vitro*		Type of PUFAs: 1. Ethyl docosahexaenoate (E-DHA). 2. Docosahexaenoic acid (DHA) 3. cis-11,14-eicosadienoic (EDA) 4. Eicosapentaenoic acid (EPA)	1. Murine RAW264.7 monocyte/macrophage cell line - [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (MTT) assay was performed to examine the effect of PUFAs on cell growth for 24 h - Tartrate-resistant acid phosphatase (TRAP) staining was performed to examine expression of osteoclast differentiation • Western blot analysis was performed to examine: • The effect of E-DHA on the role of MAPKs (ERK, JNK and p38) in Rankl signalling pathway	1. Murine RAW264.7 monocyte/macrophage cell line - MTT assay: E-DHA showed not effect on the RAW264.7 cells growth rate - TRAP staining: E-DHA decreased the maturation of preosteoclast cells most significantly and reduced the TRAP-positive multinucleated cells - Western blot: • E-DHA reduced the expression of JNK significantly but ERK, p38 and Akt were found not modulated • E-DHA significantly reduced expression of IκB • E-DHA suppressed c-Fos and NFATc1 expression	E-DHA was found to be a much more potent inhibitor of osteoclastogenesis in RANKL-induced RAW264.7 cells compared with DHA, cis-11, 14-eicosadienoic acid or EPA DHA and E-DHA can be used therapeutically to treat bone diseases, such as osteoporosis and rheumatoid arthritis

Continues TABLE 1.

Evaluation of mineral contents of *Ficus carica* for osteoporosis and rheumatoid arthritis						
Reference	Types of study	Aim of study	Subject/Sample	Methods (Parameters)	Results	Remarks/Conclusion
				• The effect of E-DHA on the NF-κB pathway via activation of IκB • The effect on the expression of c-fos and NFATc1 in RANKL-induced - Reverse transcription-polymerase chain reaction analysis (RT-PCR) was performed to examine the effects of E-DHA on the expression of osteoclast specifi c genes such as: • MMP-9 • TRAP • c-fms • β-actin (Housekeeping gene)	- RT-PCR: • E-DHA inhibits osteoclastogenesis by suppressing the MMP-9, TRAP and c-fms without changing the β-actin expression	Additional experiments will be needed to confirm its efficacy *in vivo*
			Concentration of PUFAs: 1. 0.001 μM 2. 0.01 μM 3. 0.1 μM 4. 1 μM Type of cells: 1. Murine RAW264.7 monocyte/ macrophage cell line 2. Preosteoblastic MC3T3-E1 cells	2. Preosteoblastic MC3T3-E1 cells - Alkaline phosphatase (ALP) activity assay was performed to measure the effects of 4 PUFAs on the ALP activity during osteoblast diff erentiation in MC3T3-E1 cells - RT-PCR was performed to examine the effects 4 PUFAs on the expression of osteoblast specific genes during the diff erentiation of MC3T3-E1 cells such as: • BMP2 • OCN • OPG	- ALP activity: DHA exhibited the highest ALP activity. E-DHA showed inhibition on osteoclastogenesis - RT-PCR: BMP2 was increased significantly by DHA and cis-11, 14-Eicosadienoic acid compared to the vehicle control. BMP2 was decreased by E-DHA and EPA compared to the vehicle control. OCN expression was induced slowly by E-DHA. OPG expression was consistently induced by E-DHA and cis-11, 14-eicosadienoic acid compared than DHA and EPA	
Park et al. (2009)(27)	*In vitro*		Type of *Ficus carica* fractions derived from leaves: 1. 8 types of *Ficus carica* fractions (H1-H8). HF6-FC was used most active one	- Characterization of the hexane soluble fraction of *F. carica* by GS-MS 1. Murine RAW264.7 monocyte/ macrophage cell line - MTT assay was performed to evaluate the effects of HF6-FC on cell growth for 24 h with various concentrations of HF6-FC: 1. 0.1 μg/mL 2. 1 μg/mL 3. 10 μg/mL	- Characterization of the hexane soluble fraction of *F. carica* by GS-MS: The n-hexane soluble fraction of HF6-FC exhibited the strongest anti-osteoclastogenic effect The identified components of HF6-FC: • Octadecane • Pentadecane • Hexadecane • Heptadecane • Octadecane	HF6-FC is a potent inhibitor of osteoclastogenesis in RANKL-stimulated RAW264.7 cells and in BMMs HF6-FC may potentially provide a novel therapy for disorders associated with bone loss

Continues TABLE 1.

Evaluation of mineral contents of *Ficus carica* for osteoporosis and rheumatoid arthritis						
Reference	Types of study	Aim of study	Subject/Sample	Methods (Parameters)	Results	Remarks/Conclusion
Park et al. (2009)(27)	*In vitro*		Type of cells: 1. Murine RAW264.7 monocyte/ macrophage cell line 2. Bone marrow derived macrophage	- TRAP staining was performed to examine osteoclast differentiation expression by HF6-FC - Western blot analysis was performed to examine: • The effect of HF6-FC on MAPKs (ERK, JNK, and p38) in RANKL signalling pathway • The effect of HF6-FC on NF-κB expression - RT-PCR analysis was perforemed to examine the effects of HF6-FC on: • c-Fos • NFATc1 2. Bone marrow derived macrophage - Osteoclast differentiation - TRAP staining was performed to examine osteoclast differentiation expression by HF6-FC - MTT assay was performed to evaluate the effects of HF6-FC on cell growth (data not shown)	• 2H-1-benzopyran-2-one • Nonadecane • Hexadecanoic • Acid methyl ester • Octadecanoic acid methyl este • Tridecane • Tetradecane • Eicosane • 9,12,-octadecadienoic acid methylester • 8-octadecenoic acid 1. Murine RAW264.7 monocyte/macrophage cell line - MTT assay: HF6-FC did not adversely effect on the cell growth rate of RAW264.7 at the various concentrations - TRAP Staining: Murine RAW264.7 monocyte/macrophage cell line showed TRAP-positive multinucleated osteoclasts was reducing in numbers by treatment of HF6-FC and inhibited osteoclast differentiation in a concentration-dependent manner - Western blot: • HF6-FC increased the ERK activity of RAW 264.7 cells • HF6-FC inhibited the RANKL-induced p38 kinase activation of RAW 264.7 cells • HF6-FC showed significantly reduced on the level of phosphorylated IκB-αprotein of RAW 264.7 cells • HF6-FC significantly suppressed the level of phosphorylated p65 and suppresses the NF-κB inductio by RANKL in the RAW 264.7 cells - RT-PCR: HF6-FC significantly suppresses and reduced the expression of c-Fos and NFATc1 in the RAW 264.7 cells 2. Bone marrow derived macrophage - TRAP staining: HF6-FC reduced the formation of TRAP-positive MNC in a concentration-dependent manner - MTT assay: HF6-FC did not affect the growth of BMMs (data not shown)	Further study is needed to confirm its effectiveness *in vivo*

that the E-DHA decreased the maturation of preosteoclast cells most significantly and reduced the TRAP-positive multinucleated cells in RAW264.7 cells (Choi et al. 2011). E-DHA showed no effect on the RAW264.7 cells growth rate evaluated through MTT assay after 24 h treatment (Choi et al. 2011). Western blot results showed that the E-DHA significantly reduced the expression of c-Jun NH2-terminal kinases (JNK) (Choi et al. 2011). In contrast, the extracellular signal-regulated kinase (ERK), p38 mitogen-activated protein kinases (p38) and protein kinase B (Akt) were not modulated. Treatment of E-DHA in RAW264.7 cells also significantly reduced the expression of IkappaB kinase (IκB) and suppressed c-Fos and NFATc1 expression. In addition, the increased RANKL-induced level of c-fos mRNA was reversed by E-DHA in a concentration-dependent manner. Finally, the mRNA expression of MMP-9, TRAP and c-fms was also suppressed following the treatment of E-DHA. Overall, the data suggested the inhibition of osteoclastogenesis in RAW264.7 cells by E-DHA (Choi et al. 2011). Treatment of DHA on preosteoblastic MC3T3-E1 cells has been shown to promote the highest ALP activity *in vitro*. However, after treatment of E-DHA in preosteoblastic MC3T3-E1 cells, osteoclastogenesis was inhibited. RT-PCR results showed that osteogenic markers such as BMP2 was increased significantly by DHA and EDA but decreased by E- DHA and EPA in preosteoblastic MC3T3-E1 cells. Expression of OCN was induced slowly by E-DHA compared to other PUFAs. OPG expression was consistently induced by E-DHA and cis-11, 14-eicosadienoic acid not by DHA and EPA (Choi et al. 2011).

An earlier study done by Park et al. (2009) was conducted to assess the effects of the hexane soluble fraction of *Ficus carica* leaf (HF6-FC) on RANKL-induced osteoclastogenesis in murine monocytes/macrophage RAW264.7 cells and bone marrow-derived macrophages (BMMs). In this study, they determined eight types of *Ficus carica* fractions (HF1-HF8), but only HF6-FC was the most active for all the parameters.

They showed that the n-hexane soluble fraction of HF6-FC exhibited the strongest anti-osteoclastogenic effect. They optimized the extraction method and identified components of HF6-FC which are octadecane, pentadecane, hexadecane, heptadecane, octadecane, 2H-1-benzopyran-2-one, nonadecane, hexadecanoic, acid methyl ester, octadecanoic acid methyl este, tridecane, tetradecane, eicosane, 9,12,-octadecadienoic acid methylester and 8-octadecenoic acid (Park et al. 2009).

Their sample of bone marrow-derived macrophages (BMMs) was obtained from mice tibia and femur bone marrow and MTT assay was performed to evaluate the effect of HF6- FC on the cell growth rate of RAW264.7 and BMMs at the various concentrations. The MTT showed that the HF6-FC did not adversely affect both cells. Osteoclast cells derived from BMM were successfully differentiated and cultured in medium containing M-CSF (30 ng/mL) and RANKL (200 ng/mL). The effect of HF6-FC on osteoclastogenesis in murine monocyte/macrophage RAW 264.7 cells was evaluated via TRAP staining. Treatment of HF6-FC on both cells showed a reduction in the numbers of TRAP-positive multinucleated osteoclasts indicating inhibited osteoclast differentiation in a concentration-dependent manner (Park et al. 2009). Western blot analysis showed that the HF6-FC increased the ERK activity of RAW 264.7 cells. In contrast, the HF6-FC inhibited the RANKL-induced p38 kinase activation by significantly reducing the level of phosphorylated IκB-α protein of RAW 264.7 cells. These suggested that the HF6-FC significantly suppressed the level of phosphorylated p65 and the NF-κB induction by RANKL in the RAW 264.7 cells. Reverse transcription-polymerase chain reaction analysis was performed to examine the effects of HF6-FC on expression of c-Fos and NFATc1 in the RAW 264.7 cells. The result showed that HF6-FC significantly suppresses expression of c-Fos and NFATc1 in the RAW 264.7 cells (Park et al. 2009).

Discussion

In the present review, major mineral contents of *Ficus carica*, namely potassium (K), magnesium (Mg), calcium (Ca), phosphorus (P) and Strontium (Sr) were found to be crucial for strong bone development (Khan et al. 2011; Sadia et al. 2014; Soni et al. 2014). Calcium and magnesium is a major component in bone and tooth development (Brody & Bender 1994; Reid et al. 1993; Rude et al. 2004). Potassium is a blood pressure controlling mineral and reported calcium-potassium may also neutralize increased urinary calcium loss and helping to prevent bones from thinning out at a fast rate (Cozzolino et al. 2001).

Interestingly, Strontium has been shown to improve bone health (Marie 2005; Reginster et al. 2007). Clinical studies done by Reginster et al. (2007) reported the effect of *Strontium ranelate* (PROTELOS®) was found to reduce vertebral and non-vertebral fractures in osteoporosis subjects. *Strontium ranelate* (PROTELOS®) is an oral drug for postmenopausal osteoporosis, has been reported to decrease bone resorption and to stimulate bone formation. Moreover, their finding showed that the figs is a promising source of protein, carbohydrate, fibers and vitamins, with high energy values.

Moverover, our sytematic review on molecular evaluation of *Ficus carica* showed that studies done by Choi et al. (2011) and Park et al. (2009) were focused on the effect of *Ficus carica* extract on RANKL signalling pathway which is very important for bone remodelling in bone loss and arthritis (Figure 2) (Choi et al. 2011; Park et al. 2009). Jimi et al. (2004) demonstrated that bone destruction and inflammation are closely linked in diseases via inhibition of NF-κB lead to block osteogenesis and prevent inflammatory bone destruction *in vivo*. Similar results with several studies done by Coon et al. (2007), Han et al. (2007), Mino et al. (1998) and Teitelbaum and Ross (2003), showed that the osteoclast development, involvement of inflammatory cytokines and the signalling pathway of RANKL are important in bone remodelling.

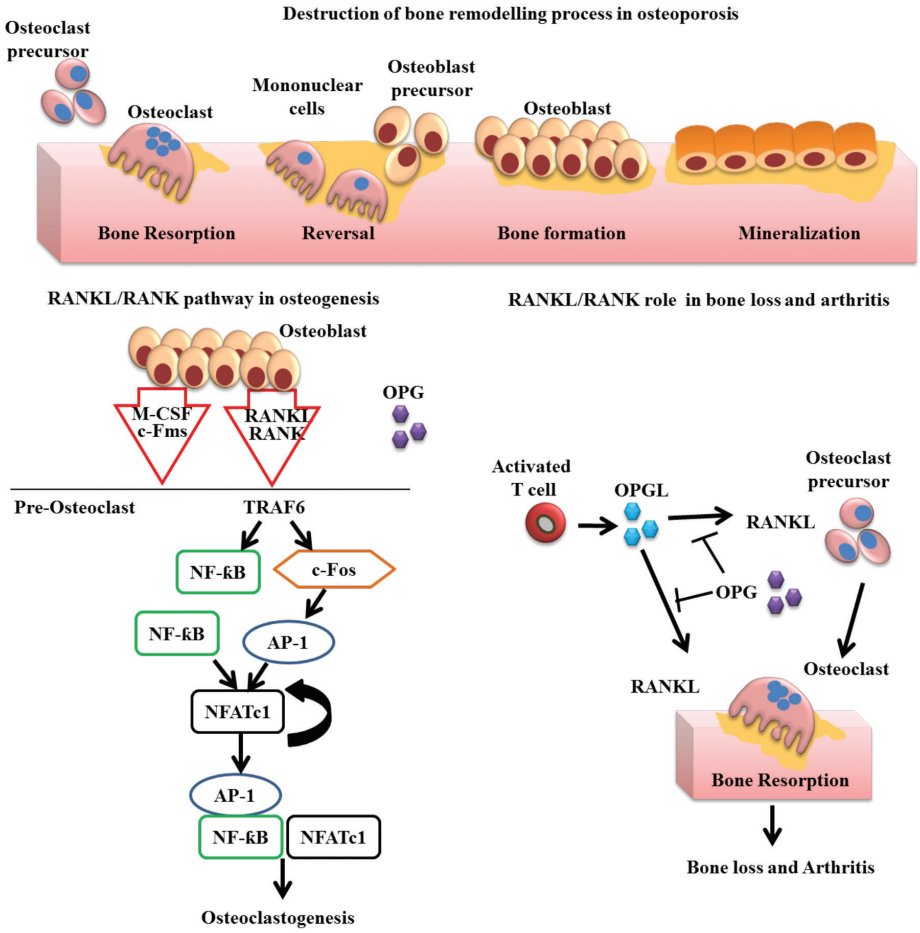

FIGURE 2. Schematic diagram destruction of bone remodelling process in osteoporosis, involving RANKL/RANK pathway

Osteoclast differentiation from monocyte/macrophage precursor cells is controlled by two currently known factors, macrophage colony-stimulation factor (M-CSF) and receptor activator of nuclear factor κB (NF-κB) ligand (RANKL) (Theill et al. 2002; Zhao et al. 2007). M-CSF and RANKL are two important cytokines that involved in osteoclastogenesis (Theill et al. 2002). The osteoblasts will secrete M-CSF to provide the survival of precursor cell signaling (Yoshida et al. 1990). RANKL binding to its receptor RANK activates TNF receptor-associated factor 6 (TRAF6), which is linked to NF-κB and mitogen-activated protein kinases (MAPKs) (Kobayashi et al. 2001; Lee et al. 2002). In addition, RANKL induces the key transcription factor for osteoclastogenesis, nuclear factor of activated T cell c1 (NFATc1) (Takayanagi et al. 2002).

In year 2005, Luo et al. showed that osteoblast lineage cells expressed a membrane bound form of RANKL after treatment with *Ficus carica* extract *in vitro*. The osteoblast precursor cell lineage, express a membrane bound form of RANKL, a member of the tumor necrosis factor (TNF) cytokine family and strongly activates the NF-κB pathway. Study done by Takayanagi et al. (2002) and Yamashita et al. (2007) reported that the binding of RANKL to its receptor RANK in bone marrow-derived macrophages (BMMs) recruits TNF receptor-associated factor 1 (TRAF) family proteins such as TRAF6, which play roles in interaction with NF-κB and c-Jun NH2-terminal kinases (JNK) pathways.

The canonical NF-κB pathway involves the phosphorylation of the IkappaB kinase (IκB) kinase complex inhibitor caused by the ligation of RANK (Luo et al. 2005). Phosphorylation of NF-κB associated IκB leads to its ubiquitination and proteosomal degradation. IκB is an enzyme complex that play role in propagating the cellular response to inflammation (Viatour et al. 2005). The transcription factor Nuclear factor of activated T- cells, cytoplasmic 1 (NFATc1), Tartrate-resistant acid phosphatase (TRAP), cathepsin K and Matrix metallopeptidase 9 (MMP-9), also plays a critical role in RANKL-induced osteoclastogenesis (Motyckova et al. 2001; Takayanagi et al. 2002). AP-1 and NF-κB binding sites were reported to be present within the promoter region of the NFATc1 gene, explaining the connection between NFATc1 and NF-κB (Zhou et al. 2002).

During osteoblast differentiation in bone remodelling, BMP-2 enhances osteoclast differentiation by upregulating the RANKL (Tachi et al. 2010). BMP-2, which belongs

to the transforming growth factor-β (TGF-β) super family, transduces its signal to the target bone genes such as alkaline phosphatase (ALP), bone sialoprotein, osteocalcin, Runt-related transcription factor 2 (RUNX2) and distal-less homeobox 5 (Dlx5) (Mukherjee et al. 2010; Tachi et al. 2010).

In this review we discussed that the E-DHA was found to be a much more potent inhibitor of osteoclastogenesis in RANKL-induced RAW264.7 cells than DHA, cis-11, 14-eicosadienoic acid or EPA done by Choi et al. (2011). Moreover, the E-DHA may exert its inhibitory effect by suppressing the JNK and NF-κB signalling pathways which correlated with the MMP-9, c-fms and TRAP expression. Interestingly, Choi et al. (2011) reported that the DHA strongly induced osteoblast differentiation in MC3T3-E1 and these results supported that a DHA diet induces bone formation but does not inhibit bone resorption in animal experiments. *In vitro* study done by Rahman et al. (2008) also reported that the effects of mixed fatty acid supplements with different DHA contents on bone mass by inhibition the RANKL- induced differentiation of osteoclasts from RAW264.7 cells after DHA treatment.

However *in vivo* study done by Poulsen et al. (2007), reported the inhibitory effects of DHA on mature osteoclasts might be minimal or transient because no effect of DHA on bone resorption was observed in growing male rats or in ovariectomized (OVX) female rats. They also reported a significantly increase in bone formation or a change in the site of bone formation.

Park et al. (2009) reported that HF6-FC inhibited NF-κB transcriptional activity, phosphorylation of IκB and p65. The involvement of these transcription factor and kinases in RANKL induced osteogenesis is well established. RANKL has been reported to activates extracellular signal-regulated kinases (ERK), JNK and Mitogen-Activated Protein Kinase p38 (p38) in osteoclasts and their precursor cells in NF-κB pathways (Chang et al. 2007; Kobayashi et al. 2001; Lee et al. 2002; Yoshida et al. 1990). However, study done by Miyazaki et al. (2000) reported that in osteoclasts differentiation, ERK activity correlates with cell survival through the activation of c-Fos, JNK increases AP-1 transcriptional activity via c-Jun phosphorylation, but not with resorption function.

Interestingly, they also showed that HF6-FC inhibited the RANKL-induced p38 kinase activation, but HF6-FC did not inhibit the ERK activation. Moreover, they showed that the HF6-FC prolonged ERK activity, suggesting the pivotal role of HF6-FC in influencing osteoclast survival through ERK, while inhibiting osteoclastogenesis via p38 kinase. Accordingly, they reported that HF6-FC did not affect the expression of MMP9, TRAP, RANK and cathepsin K in RANKL-induced RAW 264.7 cells.

STRENGTHS AND LIMITATIONS

General positive outcomes were reported in all studies included. Availability of evidence in the literature specific to the application of fig on bone health is limited. Different parts of the Ficus plant were used by investigators of each study included in the review making the interpretation of its efficacy difficult.

IMPLICATIONS FOR FUTURE RESEARCH

In order to make strong conclusions about the claimed benefits of fig on promotion bone health, further studies could help to define the minimum effective dose of fig required for beneficial effects, the minimum effective duration of consumption or supplementation, as well as the best preparation of extract for maximal beneficial effect. However, given that the evidence to date does support some impact of fig on bone health.

IMPLICATIONS FOR CLINICAL APPLICATION

The impact in *in vivo* study is unknown. If there is proven beneficial effect of fig on bone health, more work has to be initiated in order to make *Ficus carica* as a useful supplement as well as treatment for bone diseases. Nevertheless, before this could be planned, concurrently the adverse effects of fig extract must be clearly evaluated.

CONCLUSION

This study showed that *Ficus carica* has beneficial effects on bone health due to its high minerals content and inhibition of osteoclastogenesis via RANKL pathway. Therefore, *Ficus carica* has a potential to be used as a pharmaceutical product for bone health.

ACKNOWLEDGEMENTS

The authors would like to thank the Library of Universiti Kebangsaan Malaysia Medical Centre, for providing access to the databases. This study is made possible by grants from the Amrus Medik Sdn Bhd. FF-2017-020.

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Received: 1 February 2018
Accepted: 20 June 2018