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MILK-DERIVED BIOACTIVE PEPTIDES WITH ANTIOSTEOPOROTIC EFFECT: A MINI REVIEW

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ABSTRACT

Postmenopausal osteoporosis is a global health problem characterized by decreased in bone mineral density (BMD) and progressive deterioration of microarchitecture and subsequent increase in bone fragility and susceptibility to fracture. More than 200 million people suffer from osteoporosis worldwide with about 8.9 million fractures and the prevalence rate of osteoporosis is expected to increase significantly in the future because of increased in life expectancy and aging population. Milk-derived bioactive peptides from cow, goat, sheep, buffalo, and camel exhibit several potential health promoting effect including antiosteoporosis, antihypertensive, antioxidative, antithrombotic, immunomodulatory and anti-inflammatory effects. Epidemiological and intervention studies have shown that milk and milk-derived peptides prevented bone loss in pre- and postmenopausal women. Moreover, quite a lot of studies have reported that milk-derived bioactive peptides can induce osteoblast cell proliferation, differentiation and also prevented bone loss in osteoporotic rats model. Thus, milk-derived peptides exhibits beneficial effect against bone-related diseases and can be of particular interest towards prevention and management of postmenopausal osteoporosis. Hence, the present review summarizes various studies using ISI, SCOPUS and PubMed indexed journals to elucidate the potential role of milk-derived bioactive peptides with *in vitro* and *in vivo* antiosteoporotic property.

Keywords: Postmenopausal osteoporosis, Osteoblasts differentiation, Milk-derived peptides, Antiosteoporotic effect, Mechanism of action.

INTRODUCTION

Postmenopausal osteoporosis is a global health problem characterized by decreased in bone mineral density (BMD) and progressive deterioration of microarchitecture and subsequent increase in bone fragility and susceptibility to fracture (Mada *et al.,* 2017; Rachner *et al.,* 2011). The prevalence of osteoporosis is expected to rise significantly in the future because of increase in life expectancy and aging population (Kuo and Chen, 2017; Hernlund *et al.,* 2013). More than 200 million people suffer from osteoporosis worldwide and about 8.9 million fractures occurs mainly at the hip, vertebrae, and distal forearm (Minisola *et al.,* 2017), and are associated with reduced quality of life of patients, economic burden and significant morbidity and mortality (Minisola *et al.,* 2017). Several risk factors for postmenopausal osteoporosis includes aging, hormonal, and genetic factors, which are unmodifiable while life style, smoking, excessive alcohol intake, medications, and lack of exercise, are modifiable factors (Ahn and Je, 2019). Osteoporosis mainly occurs in postmenopausal women due to loss of ovarian function and estrogen deficiency during the onset of menopause (Khedgikar *et al.,* 2015; Farr *et al.,* 2013). Estrogen, a key regulator of bone metabolism is responsible for maintaining delicate balance between bone forming-osteoblast cells and bone degrading-osteoclast cells (Manolagas, 2010). Bone loss occurs due to imbalance between osteoblast and osteoclast activity infavour of osteoclast activity thereby leads to increased bone turnover mediated by receptor activator of nuclear factor-κB-ligand (RANKL) and proinflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), IL-7 and IL-1 (Muhammad *et al.,* 2018; Guillerminet *et al.,* 2010; Baek *et al.,* 2006; Bandyopadhyay *et al.,* 2006). In addition, several transcription factors such as runt-related transcription factor- 2 (Runx-2) and osterix have been identified as crucial for induction of bone forming-osteoblast cell differentiation and maturation (Wu and Lu, 2008). Bioactive peptides are specific fragments of parent protein containing 2–20 amino acids sequence (Di-Bernardini *et al.,* 2011), and can have positive health effect on body functions (Sharma *et al.,* 2011; Walther and Sieber, 2011; Shahidi and Zhong, 2008). These bioactive peptides are usually derived from the cow, goat, sheep, buffalo, and camel milk with multifunctional properties and offers a wide range of health beneficial effects such as antimicrobial, antihypertensive, antioxidative antithrombotic, immunomodulatory, anti-inflammatory and bone health promoting effects (Mada *et al.,* 2020; Kandukuri et al., 2018; Mada *et al.,* 2017; Haque *et al.,* 2009). In addition, the biological activity of these bioactive peptides depends on their amino acid composition and sequences (Sánchez and Vázquez, 2017). For example, several bioactive peptides have structural features that include the presence of hydrophobic amino acids in addition to proline, lysine or arginine groups which may confers resistant to digestion by peptidases or proteases (Kitts and Weiler, 2003). However, the present review would focus on the antiosteoporotic effect milk-derived bioactive peptides and also elucidate their possible mechanism of actions in the prevention and management of postmenopausal osteoporosis by using ISI, SCOPUS and PubMed indexed journals containing experimental reports.

PRODUCTION OF BIOACTIVE PEPTIDES

Milk has been regarded as nature's most complete food and Casein protein constitutes about 80% whereas whey accounts for the rest 20% (Phelan *et al.,* 2009; Silva and Malcata, 2005). Emerging evidences have indicated that milk is highly rich in biologically active peptides embedded in coreprotein, which

can be release by gastric digestion or fermentation process (Korhonen and Pihlanto, 2006), or through enzymatic hydrolysis (Mirzaei *et al.,* 2018; Kumar *et al.,* 2016), gastrointestinal digestion (Mohanty *et al.,* 2016), or microbial fermentation (Yahya *et al.,* 2017). The conventional and advanced methods usually involved in production of bioactive peptides were elaborated below and summarized by flow-chart (Fig.1).

Enzymatic digestion

Biologically active peptides can be produced through hydrolysis of milk protein such as casein or whey (Fig. 1) using analytical-grade proteinases such as chymotrypsin, pepsin and trypsin, individually or combined (Ugwu *et al.,* 2019; Bamdad *et al.,* 2017; Chaudhari *et al.,* 2017). The digestion of protein samples involves the use of appropriate buffer of different pH for optimum activity of hydrolytic enzymes activity. Biologically active peptides can as well be released from milk-derived proteins during gastrointestinal digestion by the action of digestive enzymes (Hernandez-Ledesma *et al.,* 2011).

Fermentation

Besides enzymatic digestion, another strategy for production of bioactive peptides is by fermentation (Fig. 1) by use of proteolytic system of microorganism (Hernandez-Ledesma *et al.,* 2014). Thus, several microorganisms can hydrolyse milk protein into peptides and amino acids which can serve as nitrogen source necessary for their growth (Juillard *et al.,* 1998). The peptides release can be isolated and purified through ultrafiltration or using molecular sieve (Palaniswamy *et al.,* 2012), and the amino acid sequences of the bioactive peptide are identified by chromatographic methods (Lin *et al.,* 2018). For instance, fermentation of milk using *Lactobacillus helveticus* and *Saccharomyces cerevisiae* releases bioactive peptides IPP and VPPP (Nakamura *et al.,* 1995). Other peptides produced by fermentation of milk with *Enterococcus faecalis* are LHLPLP and HLPLP (Quirós *et al.,* 2007).

Recombinant DNA technology

Recombinant DNA technology is also being explore for mass production of biologically active peptides (Fig. 1), especially for the synthesis of long chain peptides (Schrimpf *et al.,* 2018; De-Brito *et al.,* 2018; Chahardoli *et al.,* 2018; Boga *et al.,* 2018). Despite the advantage of possible mass production of longer peptides however, the major challenge of using recombinant DNA technique is the expression of products which may be harmful to the host. Moreover, antibacterial peptides possess strong antibacterial activity against the expression vector and comparative sensitivity to proteolytic enzymes activity (Espita *et al.,* 2009). Though, some of these limitations may possibly be overcome by expression of these bioactive peptides in the form of fusion protein or in a tandem gene that may counteract their inherent toxic properties and improve their expression levels.

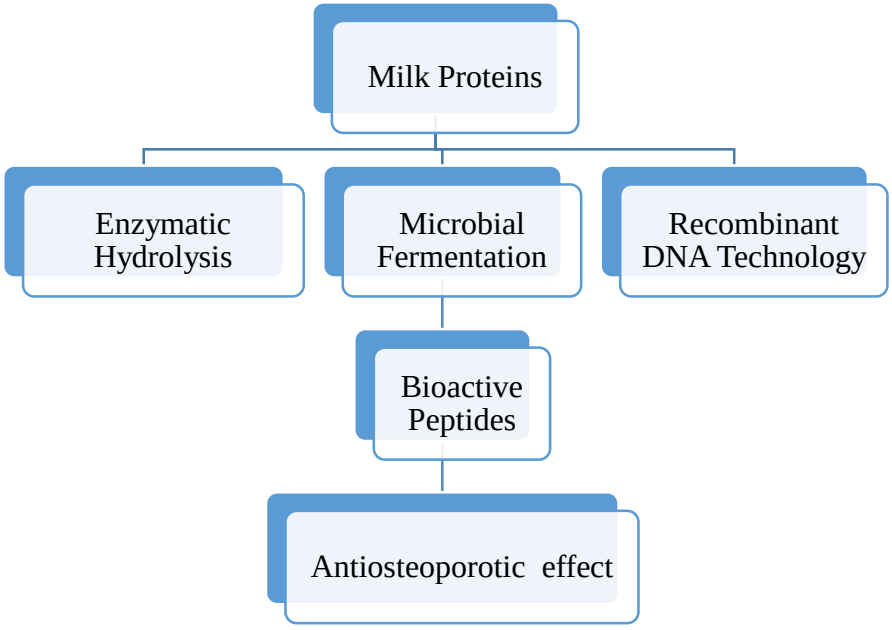

Fig. 1: Schematic representation of bioactive peptides production

DISCUSSION

Milk-derived peptides with antiosteoporotic effects in *vitro* and *in vivo*

Over the last two decades, epidemiologic and intervention studies have shown that milk and milk-derived peptides prevented bone loss in pre-and postmenopausal women (Chee *et al.,* 2003). Also, several studies thereafter have reported that milk derived bioactive peptides can induce osteoblast cells differentiation, maturation and matrix mineralization *in vitro* and thus could have beneficial effect against postmenopausal osteoporosis (Mada *et al.,* 2018; Behera *et al.,* 2013; Huttunen *et al.,* 2008). In addition, studies have reported a positive association between high dietary protein intakes with bone mineral density and fracture repair (Wengreen *et al.,* 2004), bone strength and bone formation (Huttunen *et al.,* 2007). Thus, owing to their numerous beneficial effects on humans towards prevention and management of chronic and ageing

diseases including postmenopausal osteoporosis, bioactive peptides are now being considered as the new generation of biologically active regulators (Lemes *et al.,* 2016). For instance, casein phosphopeptide have been demonstrated to enhance vitamin D-independent bone calcification in rachitic infants (Mellander, 1950), and Ca-bound casein phosphopeptide prevent bone loss in OVX rats model (Tsuchita *et al.,* 1996). Moreover, fermented milk containing bioactive peptides IPP and VPP (Table 1), produced by fermentation of milk with *Lactobacillus helveticus* can enhance BMD in growing rats (Narva *et al.,* 2004a; Narva *et al.,* 2004b). Furthermore in another study, long-term treatment of in human preosteoblast cells (MSCs) with IPP peptide improved matrix mineralization due to enhance cell survival (Behera *et al.,* 2013). In addition, bioactive peptides with antioxidant and osteoblast proliferation activity were obtained from buffalo milk casein hydrolysate by pepsin–trypsin hydrolysis (Reddi *et al.,* 2016a). Among these peptides, NAVPITPTL peptide markedly increased osteoblast cells differentiation via activation of pAkt signaling pathway (Reddi *et al.,* 2016b). Besides, peptide VLPVPQK exhibited antiosteoporotic effects via inhibition of oxidative damage and bone-resorbing cytokines production in OVX rats as demonstrated in Table1 (Reddi *et al.,* 2019; Huttunen *et al.,* 2008). The proposed possible mechanisms of bioactive peptides against postmenopausal osteoporosis have been shown in Fig. 2.

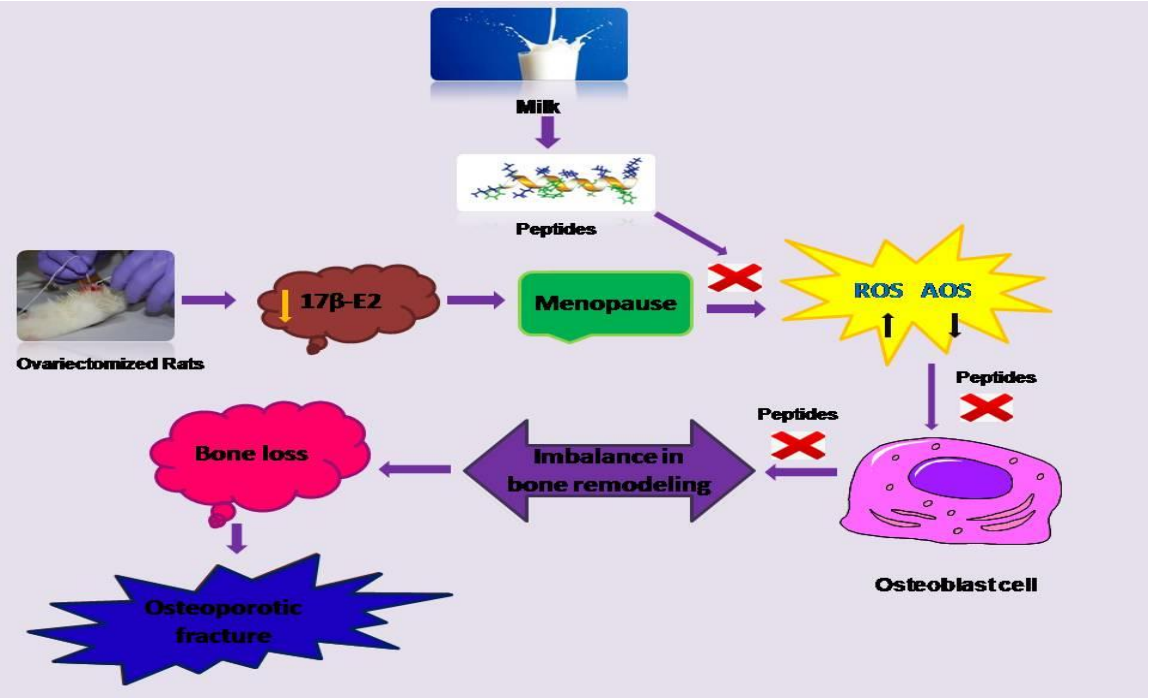

Fig. 2: The proposed possible mechanisms of bioactive peptides against postmenopausal osteoporosis. Abbreviation: 17β-E2: 17β-Estradioal, ROS: Reactive oxygen species, AOS: Antioxidant system.

Table 1: Milk-derived bioactive peptides with *in vitro* and *in vivo* antiosteoporotic effect

Bioactive peptide	Treatment	Outcome	Reference
IPP, VPP and LKP	hMSCs was treated with peptides at dose of 5, 50 and 500 µM for 6, 24 and 48 hr respectively.	IPP increase hMSC proliferation, viability and differentiation into matured osteoblast via up-regulating BMP-2, BMP-5, Parathyroid hormone-related genes (PTHrP) expression and down-regulation of Caspase-8 and vitamin D receptor genes expression.	Huttunen *et al.,* 2007
	Long-term treatment of hMSCs with IPP, VPP and LKP at dose of 50 µM for 14 days.	However, VPP and LKP had modest influence on osteoblast gene expression.IPP increased mineral formation due to enhanced cell survival and matrix formation. Also, increases Runx2 gene expression, and decreased Caspase-8 activity and RANKL/OPG ratio but has no effect on ALP activity.	Huttunen *et al.,* 2008
NAVPITPTL	Treatment of peptide to calvarial osteoblast cells at a dose of 30ng/ml for 21 days.	Elevated mineralization and increase ALP activity. Also up regulated the expression of Osteocalcin, Collagen (type I) and Alkaline phosphatase genes via phosphorylation of AKT.	Reddi *et al.,* 2016a
VLPVPQK	Treatment of calvarial osteoblast cells with antioxidative peptide at dose of 50-200ng/ml for 7, 14, and 21 days.	Increases osteoblast cell viability, enhanced matrix mineralization and unregulated osteoblast cells differentiation marker (ALP, OCN, COL-I) genes expression and decreases bone resorbing and inflammatory cytokines expression.	Mada *et al.,* 2017a; Mada *et al.,* 2017b
Casein phosphopeptides (CPP)	Treatment of OVX rats with Ca-bound and Ca-free CPP for 17 weeks provided 62.5 and 100% of dietary phosphorus	Increases Bone Mineral Density (BMD) in OVX rats.	Tsuchita *et al.,* 1996
***Lactobacillus helveticus* fermented milk containing peptides IPP and VPP**	Treatment of Postmenopausal women with fermented milk containing 14.5 mg/ 100 g milk of IPP and VPP together or control milk.	Fermented milk containing IPP and VPP reduces serum PTH level and increase serum calcium level.	Narva *et al.,* 2004a; Narva *et al.,* 2004b
VLPVPQK	Treatment of OVX rats with peptide at dose of 50 and 100µg/kg/day for 8 weeks.	Increases Bone Mineral Density (BMD) and improved trabecular microarchitecture of femur in OVX rats and also augmented biomechanical bone strength and inhibited bone resorption markers in OVX rats	Mada *et al.,* 2018
NAVPITPTL	Feeding of OVX rats with peptide at a dose of 100µg/kg/day for 8 weeks.	Enhanced femur BMD and microarchitecture. In addition to improved biomechanical bone strength and suppressed RANKL gene expression in OVX rats.	Reddi *et al.,* 2019

CONCLUSION

In conclusion, the present review describes the potential antiosteoporotic effect of milk-derived bioactive peptides with osteoblast cells differentiation, matrix mineralization and improvement of bone mineral density in osteoporotic rats model. Although, these bioactive peptides could stimulate new bone formation by acting on different signaling pathways linked to bone formation. Altogether bioactive peptides may have beneficial effect against bone-related disorders including postmenopausal osteoporosis.

ABBREVIATIONS

OVX, ovariectomy; OVX rats, ovariectomized rats; BMD, bone mineral density; RANKL, receptor activator of nuclear factor-κB ligand; IL-1, interleukin-1; IL-6, interleukin-6; IL-7, interleukin-7; TNF-α, tumor necrosis factor-α; M–CSF, macrophage-colony stimulating factor; Runx-2, runt-related transcription factor-2.

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CONFLICT OF INTERESTS

The authors declare that they have no conflicts of interest with the contents of this article.

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