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 antosteoporosis, 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 antosteoporotic 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 (Khedigkar 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 in favour of osteoclast activity thereby leads to increased bone turnover mediated by receptor activator of nuclear factor-kB 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; Bandypadhyay 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 released 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 (Quiros 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.

**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 antiosteoerotic 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. 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., 2007          |
| 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.
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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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