Vietnam Journal of Science and Technology ***64***(1) (2026) 37–46
DOI: https://doi.org/10.15625/2525-2518/18785

Rutin exhibits an anti-resorptive effect in a medaka fish model of osteoporosis

To Thanh Thuy[1,*], Nguyen Thi Bich Diep[1], Nguyen Tuong Anh[1], Nguyen Huy Manh[1], Phuong Thien Thuong[2]

[1]*VNU University of Science, No. 334 Nguyen Trai, Thanh Xuan, Ha Noi 100000, Viet Nam*
[2]*Biotechnology Division, Vietnam-Korea Institute of Science and Technology, Hoa Lac Hi-Tech Park, km 29, Thang Long Boulevard, Hoa Lac ward, Ha Noi, Viet Nam*

[*]Email: *tothanhthuy@hus.edu.vn*

Received: 31 August 2023; Accepted for publication: 26 March 2025

Abstract. With the increasing prevalence of osteoporosis worldwide due to an aging population, there is a substantial need for the search and development of new anti-osteoporosis substances. Rutin (quercetin-3-*O*-rhamnosyl glucoside) is a flavonoid glycoside found in many plants and herbal medicines, known for its potent antioxidant and potential osteoprotective properties. In this study we investigated the anti-osteoporotic effects of rutin, for the first time, using a medaka fish (*Oryzias latipes*) model for osteoporosis. The medaka fish model is a non-mammalian model that is increasingly preferred for drug screening. RANKL-induced osteoporosis fish larvae were treated with rutin at five different doses (10, 25, 50, 100, and 200 µM) for 96 hours starting from 7 days post-fertilization (dpf). The effect of rutin on bone damage was assessed via indexes of mineralization protection (I_P) which are based on the index of bone mineralization (I_M) of the tested fish. The results showed that rutin significantly reduced the level of RANKL-induced bone damage at concentrations of 10, 25, 50, and 100 µM, with the highest effect observed at a concentration of 10 µM. These findings provide important evidence for further studies on the bone-protective effects of rutin on medaka fish models for the development of anti-osteoporosis drugs.

Keywords: medaka fish, osteoporosis, I_M, I_P, rutin.

Classification numbers: 1.1.1,1.2.1.

1. INTRODUCTION

Bone is a dynamic organ that undergoes continuous repair and renewal through a remodeling process, in which osteoclasts resorb old bone and osteoblasts form new bone. The coordinated activity of these cells maintains bone homeostasis. Excessive bone resorption or impaired bone formation can lead to osteopenia and osteoporosis [1].

Osteoporosis is a common bone disease affecting primarily the elderly and postmenopausal women, characterized by low bone mass and an increased risk of fractures. Osteoporosis-related fractures occurred in one in three women and one in five men over 50 years of age worldwide, imposing a substantial societal burden [2, 3]. In Viet Nam, osteoporosis prevalence reached 27 % in women and 13 % in men over 50, highlighting a significant public health concern [4].

Bisphosphonates, such as alendronate and risedronate, are widely used as first-line therapies for osteoporosis [5, 6]. Although current anti-osteoporosis drugs inhibit bone resorption and/or stimulate bone formation, their efficacy and safety remain limited, highlighting the need for new osteoprotective agents [3].

Receptor activator of nuclear factor kappa-B ligand (RANKL), a member of the TNF superfamily, is a key regulator of osteoclast differentiation [7]. Elevated RANKL expression promotes bone resorption and is associated with both primary and secondary osteoporosis [5, 7, 8]. Therefore, RANKL-induced animal models are valuable for studying osteoporosis.

Medaka and zebrafish are valuable non-mammalian models for bone disease research [9]. The *rankl*:HSE:CFP transgenic medaka developed by To et al. [10] exhibits heat-shock-induced Rankl expression, leading to osteoclastogenesis and an osteoporosis-like phenotype. In this study, sublines showing bone damage restricted to neural arches [11, 12] were used to evaluate the anti-osteoporotic effect of rutin. Bone mineralization was quantified using the index of mineralization (I_M), based on the length of mineralized neural arches, from which the indices of bone damage (I_D) and bone protection by tested substances (I_P) were calculated [13].

Rutin (quercetin-3-*O*-rutinoside) (Figure 1) is a natural flavonoid glycoside readily extracted in Viet Nam from "hòe hoa" - *Styphnolobium japonicum* (L.) Schott, "mạch ba góc" - *Fagopyrum esculentum* Moench, and "bát giác liên" - *Podophyllum tonkinense* Gagnep [14, 15]. It possesses strong antioxidant and diverse pharmacological activities and has shown osteoprotective potential in previous studies [16–19]. Here, we evaluated for the first time the anti-resorptive effect of rutin using the *rankl*:HSE:CFP transgenic medaka osteoporosis model.

2. MATERIALS AND METHODS

2.1. Materials

The *rankl*:HSE:CFP transgenic fish [10] were maintained in our laboratory. Rutin (quercetin-3-*O*-rutinoside) (Figure 1), isolated from *Podophyllum tonkinense* Gagnep, had a purity of 96% as determined by high-performance liquid chromatography (HPLC) [20]. DMSO (Dimethyl sulfoxide) and other reagents were commercially obtained.

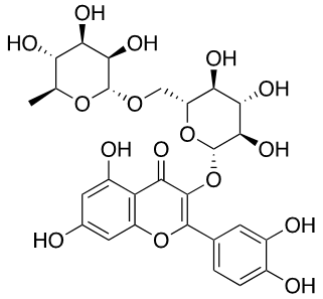

Figure 1. Chemical structure of rutin.

2.2. Methods

2.2.1. Fish lines and fish maintenance

The d1d1 subline of *rankl*:HSE:CFP transgenic fish (hereafter named Rankl fish/embryo(s)/larva(e)) was crossed with wild-type fish to obtain hemizygous Rankl offspring

for anti-resorptive evaluation [10, 11]. Fish and embryos were maintained under standard conditions using E3 (5 mM NaCl, 0.17 mM KCl, 0.33 mM $CaCl_2$, and 0.33 mM $MgCl_2$) and 0.03% sea salt media as previously described [10-13]. All experiments were approved by the Dinh Tien Hoang Institute of Medicine, Ha Noi, Viet Nam (IRB-AR.002).

2.2.2. Rutin treatment

Rutin treatment was performed as previously described [13]. Hemizygous Rankl larvae (n = 30/group) were treated with rutin (10, 25, 50, 100, or 200 µM, with a final DMSO concentration of 0.25 %), 0.25% DMSO (solvent control), alendronate (Sigma A4978; 25 µg/mL, positive control), or E3 (untreated control) from 7 to 11 days post fertilization (dpf. Heat shock was applied at 39 °C for 90 min at 9 dpf, and wild-type fish were used as a normal control.

2.2.3. The I_M method for bone mineralization quantification

At 11 dpf, fish larvae were fixed in 4 % PFA and stained with 0.5 % alizarin red to visualize mineralized bone, as previously described [10, 21]. Specimens were imaged at 10× magnification using a Zeiss Axioplanz microscope equipped with an Optika B3 Camera, and images were analyzed with ImageJ software.

Mineralized bone damage in neural arches (as indicated by green arrows in Figure 2) was a characteristic feature of Rankl fish [10–12]. The lengths of the first 15 mineralized neural arches were measured to calculate the index of bone mineralization (I_M), defined as the sum of individual arch lengths and calculated as follows:

$$I_M = \sum_{k=1}^{15} L \quad (1)$$

where 'k' represents the ordinal number and 'L' is the length of each arch [13]. I_M is inversely correlated with bone damage, with lower I_M values indicating greater mineralized bone loss.

When the I_M of wild-type ($I_{M(WT)}$) and of Rankl fish group ($I_{M(Rankl)}$) are determined, the index of mineralization damage (I_D) of the Rankl fish group can be calculated by the following formula:

$$I_D = \frac{I_{M(WT)} - I_{M(Rankl)}}{I_{M(WT)}} x\ 100\ \% \quad (2)$$

The index of bone mineralization protection (I_P) of a tested substance can be calculated based on I_M by the following formula:

$$I_P = \frac{I_{M(+Rankl+S)} - I_{M(+Rankl-S)}}{I_{M(-Rankl-S)}} x\ 100\ \% \quad (3)$$

where "± Rankl" indicates with/without Rankl; "± S" indicates with/without tested substance. The formula reflects the anti-resorptive efficacy of the tested compound, where I_P denotes the percentage of bone protected by rutin.

2.2.4. Statistical analysis

Statistical analyses were performed using Prism 9 software (GraphPad Software Inc., San Diego, CA) with unpaired Student's t-test or one-way ANOVA followed by Tukey's test. Data are presented as mean ± S.E.M., and differences were considered significant at $p < 0.05$ (marked with one asterisk (*)), $p < 0.01$ (**), $p < 0.001$ (***), or $p < 0.0001$ (****).

3. RESULTS AND DISCUSSION

3.1. Osteoporosis-like phenotype of d1d1 *rankl*:HSE:CFP larvae

Since intergenerational variation over time in expression and function of a transgene is an existing concern in transgenic animal-based research [22], we first checked whether the larval offspring of the d1d1 Rankl fish ensure suitable phenotypes for drug treatment. Rankl larvae were heat-shocked at 39 °C for 90 minutes at 9 dpf, then fixed and stained with alizarin red at 11 dpf. The first fifteen vertebrae were then captured and their representative images are presented in Figure 2.

As expected, while their wild-type (WT) siblings had intact bone structures (yellow arrow in Figure 2A), all d1d1 Rankl larvae showed partly or complete destruction in many mineralized neural arches (green and light green arrows in Figure 2B). The bone mineralization levels of fish larvae from the two groups (n = 15 for each group) were then determined by the mean values of the mineralization Indexes I_M, which were 2400.30 (WT) and 891.16 (Rankl), respectively. Student's t test was used to compare these groups with p-value < 0.0001 (Figure 2C). Afterward, the Index of bone mineralization damage I_D of the Rankl fish was calculated to be 62.87 %, meaning these larvae had lost about 62.87 % of their mineralized neural arches (Figure 2D). Moreover, the homogeneity observed in the bone destructed phenotype of these Rankl-induced larval individuals indicated that the d1d1 fish line was suitable for experiments in this study.

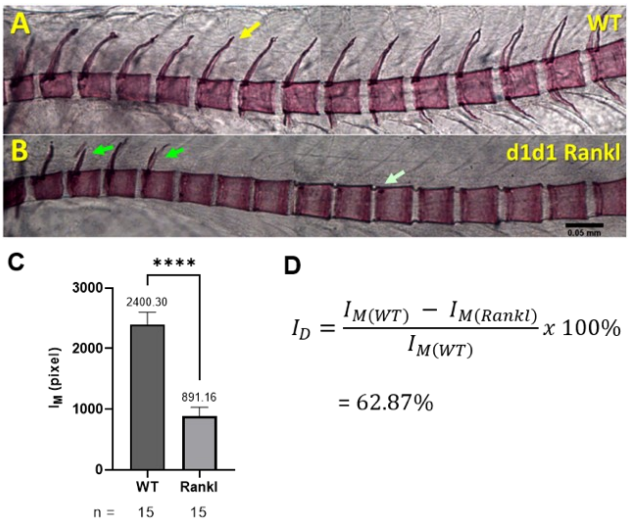

Figure 2. Images of mineralized-structure staining of the first 15 vertebrae of a wild-type (WT) larva (A) and a d1d1 *rankl*:HSE:CFP larva (B). Yellow arrows indicate intact neural arches, green arrows indicate partly destructed neural arches, and light green ones indicate completely destructed neural arches. (C) Mean values of mineralization index (I_M) of WT and Rankl fish; n: number of fish in each corresponding group; (****) p < 0.0001. (D) Index of mineralization damage of d1d1 Rankl fish.

3.2. Rutin shows anti-resorptive effect at doses of 10, 25, 50, and 100 µM in Rankl-induced osteoporotic larvae

To assess the ability of rutin to protect mineralization structures from Rankl-induced bone damage, we first tested rutin at doses of 25 and 100 µM, chosen based on previous *in vitro* studies

[18, 23-26], and then expanded the concentration range by adding two polar concentrations and an intermediate concentration (10, 200, and 50 µM, respectively).

Representative images of alizarin red-stained fish in the treated and control groups are shown in Figure 3. While mineralized bone structures remained intact in the wild-type larvae of all groups, all Rankl larvae showed damage in neural arches at various levels. Among the Rankl larvae of the DMSO control groups, the most severe destruction was observed, with fractured, shortened, or completely destructed arches. Statistical analysis revealed that the mean I_M values, which represent the level of mineralization in neural arches of the DMSO groups, fluctuated slightly in the three experiments (n = 30 in each group for one experiment; I_M = 801.36, 819.51, and 707.39 for DMSO groups of experiments 1, 2, and 3, respectively). Lateral images of the non-treated group (E3) exhibited a similar pattern of bone loss phenotype as those of the DMSO group. This was statistically confirmed when no significant difference was found between these groups, indicating that 0.25 % DMSO did not affect bone mineralization in this experimental setting.

Larvae treated with rutin at doses of 25 and 100 µM appeared to have milder bone damage than their corresponding non-treated or solvent control (Figure 3B-C). One-way ANOVA statistical analysis revealed that the mean I_M of the 25 µM Rutin-treated Rankl fish (1092.13, n = 30) is significantly higher than the DMSO (1) and E3 (1) groups (801.36, n = 30, $p < 0.05$ and 713.28, n = 30, $p < 0.01$, respectively). Moreover, 100 µM rutin-treated larvae also had a mean I_M value (1356.4, n = 30) significantly higher than that of both the DMSO(2)-treated group and the E3(2) group (819.51 and 833.69, respectively, $p < 0.05$) (Figure 3B'-C'). Thus, rutin at doses of 25 and 100 µM reduces the level of mineralized bone damage in the transgenic *rankl*:HSE:CFP osteoporosis fish model.

Next, we proceeded to assess the impact of rutin at concentrations of 10, 50, and 200 µM alongside alendronate, which served as a positive control (n = 30 for each group). Alendronate has been widely employed as a standard drug in the treatment of osteoporosis and has been shown to exhibit potent anti-resorptive effects at a dosage of 25 µg/mL in this Rankl-induced fish model [15, 18]. Among the Rankl groups, the alendronate-treated group exhibited the least destruction in neural arches and the highest index of mineralization value (I_M = 1113.73, $p < 0.001$). Following this, the group treated with rutin at a concentration of 10 µM (I_M = 1043.29, $p < 0.05$) and rutin at a concentration of 50 µM (IM = 957.73, $p < 0.05$) showed comparatively lower damage. Interestingly, the highest dose of rutin (200 µM, I_M = 700.18) appeared to have no effect on bone resorption, as evidenced by a comparable damage pattern to the DMSO(3) and E3(3) control groups, whose I_M values were 707.39 and 635.12, respectively. This observation was further supported by statistical analysis (Figure 3D-D').

Taken together, our results indicate that rutin has the potential to decrease damage to mineralized structures in Rankl fish at four different doses (10, 25, 50, and 100 µM). The effectiveness of rutin at these doses, along with alendronate, was compared using the index of mineralization protection (I_P) shown in Figure 3E. Alendronate at 25 µg/mL demonstrated the highest I_P value of 23 %. Interestingly, rutin at the lowest concentration of 10 µM exhibited a higher I_P value (19 %) compared to all other rutin doses (18 %, 14 %, and 17 % for 25, 50, and 100 µM rutin, respectively). Taking into consideration both cost and treatment effectiveness, it is recommended that further experiments investigate the underlying mechanism of rutin's anti-osteoporosis ability using a dose of 10 µM.

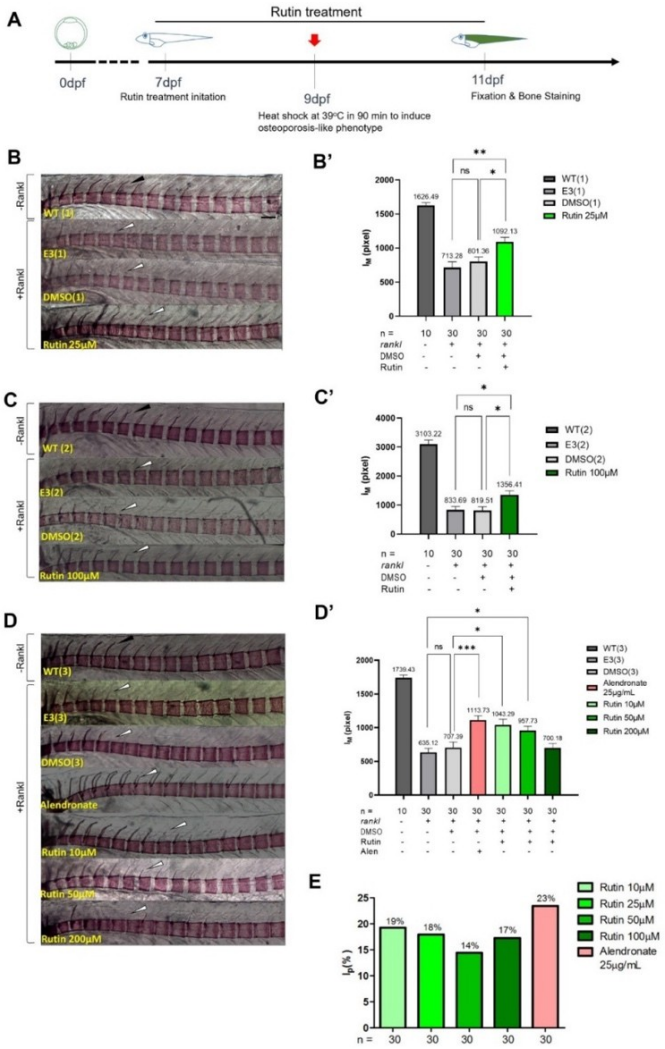

Figure 3. Rutin protects mineralized bone of neural arches from resorption in Rankl-induced osteoporotic fish. (A) Rutin treatment procedure with red arrow indicating heat-shock induction and fish in green symbolizing one with heat-shock induced Rankl. (B-B') Representative image of fish treated with 25 μM rutin, DMSO(1), E3(1), and WT(1) control groups and corresponding mean values of mineralization index (I_M). (C-C') Representative image of fish treated with 100 μM rutin, DMSO(2), E3(2), and WT(2) control groups and corresponding mean values of mineralization index (I_M). (D-D') Representative image of fish treated with 25 μg/mL alendronate, 10, 50 and 200 μM rutin, DMSO(3), E3(3) and WT(3) control groups and corresponding mean values of mineralization index (I_M). Black arrowheads indicate intact bone structure while white arrowheads indicate damaged neural arches. n: number of fish in corresponding group. + or – indicates the presence or absence of corresponding factors written on the left side for each fish group. +/-Rankl indicates fish larvae with or without ectopic Rankl expression. (*) $p<0.05$; (***) $p<0.001$. Bars indicate S.E.M. Scale bar: 0.05 mm. (E) Index of mineralization protection (I_P) of rutin at effective doses and alendronate.

3.3. Discussion

The findings of this study demonstrate that rutin, when administered at doses of 10, 25, 50, and 100 μM, can reduce mineralized bone damage in a Rankl-induced medaka fish model of

osteoporosis. The bone protection index for rutin at these doses was observed to be 19 %, 18 %, 14 %, and 17 %, respectively. As a positive control, alendronate, a standard anti-osteoporosis drug, at a concentration of 25 µg/mL, exhibited a protective index value of 23 %. It is worth noting that the highest tested dose of rutin, at 200 µM, did not display any significant effects. Therefore, the anti-resorptive effect of rutin in this fish osteoporosis model is demonstrated to be dose-dependent.

The fact that rutin is effective at lower doses but not at the highest tested dose is explainable. Bioactive substances may not exhibit efficacy at high doses due to either a hormetic effect, where high doses can cause toxic effects on the tested organisms [26], or receptor saturation resulting in a saturation effect [27]. When compared to the effect of alendronate, which at a concentration of 25 µg/mL is equivalent to 75 µM (based on the molecular weight of alendronate - Sigma A4978 is 325.12), rutin demonstrates a lower bone protective effect but at lower doses.

With the increasing number of people worldwide affected by osteoporosis due to an aging population, the search for bone-protective compounds is of utmost importance in the development of better drugs and medications. Rutin has been reported to possess a wide range of biological abilities, including anti-oxidative, anti-viral, and anti-inflammatory properties [14]. Several studies conducted on cell and mouse models have demonstrated the inhibitory effect of rutin on osteoclast activity by reducing the expression of key elements in NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) signaling pathway, such as TNF-α (tumor necrosis factor alpha), IL-1β (interleukin-1 beta), and IL-6 [17, 18, 27]. Furthermore, a study by Xiao *et al.* [19] in 2019 suggested the role of rutin in down-regulating FNCD1 (fibronectin type III domain-containing protein), which may protect against trabecular bone loss in ovariectomized mice and induce bone marrow mesenchymal stem cell autophagy via the Akt/mTOR signaling pathway. This data provides valuable evidence for further investigation of rutin as a potential bone-protective agent.

Apart from various mammalian animals, medaka (as well as zebrafish) has emerged as a highly effective non-mammalian model for studies in the field [28, 29]. Our study represents one of the initial reports on this particular fish model, providing evidence that supports previous research findings. Additionally, our fish model is induced by the overexpression of Rankl, a key factor whose increased expression is linked to both primary and secondary osteoporosis [7]. Thus, the finding that rutin can lessen Rankl-induced bone damage suggests its potential applicability in the treatment of both types of osteoporosis. However, the mechanisms that underlie the anti-resorptive effect of rutin in this fish model remain to be further elucidated. Furthermore, as the osteoporosis phenotype of the fish was induced by increased Rankl, rutin may likely inhibit the Rankl/Rank pathway. Consequently, it is suggested that the analysis of factors involved in these pathways, including TRAF6 (tumor necrosis factor receptor associated factor 6), NFATc1 (nuclear factor of activated T-cells, cytoplasmic 1), and c-Fos [6], be conducted to investigate the molecular mechanisms underlying rutin's effects.

Rutin has also been reported to exhibit bone anabolic potential by promoting osteoblast formation and mineralization *in vitro* [23, 25, 30]. These findings suggest the need for further investigation using medaka fish models to explore the potential bone anabolic effects of this substance.

The mode of how rutin can penetrate medaka fish larvae aged 7 to 11 days requires further investigation. However, absorption through the skin and via ingestion are the two probable routes of drug absorption, as reported in zebrafish larvae, a similar fish model to medaka [31, 32]. To understand the pharmaceutical effects of bioactive substances, it is important to understand their

metabolism and bioavailability in tested animals. Rutin, when orally administered to rats, has been reported to be metabolized into quercetin sulfates and quercetin glucuronides, and to be present in the blood [33]. However, there are still no studies on the metabolism and bioavailability of rutin in fish in general or in medaka fish in particular, suggesting that this issue needs to be elucidated. This is a relatively new area of research, and further studies are warranted to compare how medaka fish metabolize active natural compounds compared to those used in standard *in vivo* testing. Since many natural compounds require metabolism to become active (prodrugs), while others become inactive after this process, understanding these variations across models is crucial. While the medaka fish shares many similar biological processes with mammals [8, 9], confirmation through metabolic studies would strengthen its validity as a reliable model that correlates well with commonly used animals.

4. CONCLUSIONS

Rutin (quercetin-3-*O*-rutinoside) exhibited an anti-resorptive effect in the *rankl:*HSE:CFP medaka fish model of osteoporosis at doses of 10, 25, 50, and 100 µM, resulting in the preservation of 18 %, 14 %, and 17 % of the bone against resorption, respectively. This study provides the first evidence of the bone protective potential of the compound in a non-mammalian model. These findings suggest the need for further research using medaka models to explore the potential of rutin for drug development in the treatment of osteoporosis.

Acknowledgements. We would like to thank Pham Thi Thanh, Pham Van Cuong, Lai Thi Thuy, and other members from the BoneMed laboratory for their kind support; Dr. Tran Duc Long and Assoc. Prof. Vu Thi Thu for precious and constructive advice; We are also grateful to Assoc. Prof. Nguyen Lai Thanh, CELIFE, and the staff of the Faculty of Biology, VNU University of Science for their assistance in microscopic techniques. This study was supported by the NAFOSTED research fund under grant number 106-YS.06-2014.15.

CRediT authorship contribution statement. To Thanh Thuy: Methodology, Investigation, Writing – original draft, Writing – review & editing, Funding acquisition, Supervision. Nguyen Thi Bich Diep: Methodology, Investigation, Writing – original draft. Nguyen Tuong Anh: Formal analysis, Writing – original draft. Nguyen Huy Manh: Formal analysis, Investigation. Phuong Thien Thuong: Methodology, Writing – review & editing.

Declaration of competing interest. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

REFERENCES

1. Al-Bari A. A., Mamun A. A. - Current advances in regulation of bone homeostasis. FASEB BioAdv., **2** (2020) 668-679. https://doi.org/10.1096/fba.2020-00058.
2. International Osteoporosis Foundation - Osteoporosis. https://www.osteoporosis.foundation/educational-hub/topic/osteoporosis (accessed 2023/06/25).
3. Khosla S., Hofbauer L. C. - Osteoporosis treatment: recent developments and ongoing challenges. Lancet Diabetes Endocrinol., **5** (2017) 898-907. https://doi.org/10.1016/S2213-8587(17)30188-2.
4. Hoang D. K., Doan M. C., Mai L. D., Ho-Le T. P., Ho-Pham L. T. - Burden of osteoporosis in Vietnam: An analysis of population risk. PLoS One, **16**(6) (2021) e0252592. https://doi.org/10.1371/journal.pone.0252592.

5. Tu K. N., Lie J. D., Wan C. K. V., Cameron M., Austel A. G., Nguyen J. K., Van K., Hyun D. - Osteoporosis: A review of treatment options. Pharm. Ther., **43** (2018) 92-104.
6. Sharpe M., Boble S., Spencer C. M. - Alendronate: an update of its use in osteoporosis. Drugs, **61** (2001) 999-1039. https://doi.org/10.2165/00003495-200161070-00010.
7. Boyce B. F., Xing L. - The RANKL/RANK/OPG pathway. Curr. Osteoporos. Rep., **5** (2007) 98-104. https://doi.org/10.1007/s11914-007-0024-y.
8. Cheng C. H., Chen L. R., Chen K. H. - Osteoporosis due to hormone imbalance: An overview of the effects of estrogen deficiency and glucocorticoid overuse on bone turnover. Int. J. Mol. Sci., **23** (2022) 1376. https://doi.org/10.3390/ijms23031376.
9. Lleras-Forero L., Winkler C., Schulte-Merker S. - Zebrafish and medaka as models for biomedical research of bone diseases. Dev. Biol., **457** (2020) 191-205. https://doi.org/10.1016/j.ydbio.2019.07.009.
10. To T. T., Witten P. E., Renn J., Bhattacharya D., Huysseune A., Winkler C. - Rankl-induced osteoclastogenesis leads to loss of mineralization in a medaka osteoporosis model. Development, **139** (2012) 141-150. https://doi.org/10.1242/dev.071035.
11. Cuong P. V., Thanh P. T., Hoa N. T., Long T. D., Thuy T. T. - Segregation of rankl:HSE:CFP medaka transgenic fish line for use as osteoporosis models. VNU J. Sci. Nat. Sci. Technol., **31** (2015) 24-34.
12. Thuy L. T., Thanh P. T., Cuong P. V. C., Long T. D. L., Thuy T. T. - Stability of the transgene rankl in the RANKL:HSE:CFP transgenic medaka fish used as a model for osteoporosis. Vietnam J. Physiol., **19** (2015) 10-17.
13. Cuong P. V., Thanh P. T., Thuy L. T., Dat T. C., Huong N. V. M., Tam H. T. T. M., Thuong P. T. T., Long T. D., Winkler C., Thuy T. T. - Icariin reduces bone loss in a Rankl-induced transgenic medaka (Oryzias latipes) model for osteoporosis. J. Fish Biol., **98** (2021) 1039-1048. https://doi.org/10.1111/jfb.14241.
14. Martiniakova M., Babikova M., Mondockova V., Blahova J., Kovacova V., Omelka R. - The role of macronutrients, micronutrients and flavonoid polyphenols in the prevention and treatment of osteoporosis. Nutrients, **14**(3) (2022) 523. https://doi.org/10.3390/nu14030523.
15. Paniwnyk L., Beale E., Lorimer J. P., Mason T. J. - The extraction of rutin from flower buds of Sophora japonica. Ultrason. Sonochem., **8** (2001) 299-301. https://doi.org/10.1016/s1350-4177(00)00075-4.
16. Patel K., Patel D. K. - Chapter 26 - The beneficial role of rutin, a naturally occurring flavonoid in health promotion and disease prevention: A systematic review and update. In *Bioactive Food as Dietary Interventions for Arthritis and Related Inflammatory Diseases*, Academic Press (2019) 457-479.
17. Lee H. H., Jang J. W., Lee J. K., Park C. K. - Rutin improves bone histomorphometric values by reduction of osteoclastic activity in osteoporosis mouse model induced by bilateral ovariectomy. J. Korean Neurosurg. Soc., **63** (2020) 433. https://doi.org/10.3340/jkns.2019.0097.
18. Kyung T. W., Lee J. E., Shin H. H., Choi H. S. - Rutin inhibits osteoclast formation by decreasing reactive oxygen species and TNF-alpha by inhibiting activation of NF-kappaB. Exp. Mol. Med., **40** (2008) 52-58. https://doi.org/10.3858/emm.2008.40.1.52.
19. Xiao Y., Wei R., Zhen Y., Lan X., Kuang J., Hu D., Song Y., Luo J. - Rutin suppresses FNDC1 expression in bone marrow mesenchymal stem cells to inhibit postmenopausal osteoporosis. Am. J. Transl. Res., **11** (2019) 6680-6690.
20. Dung N. T., Long M. T., Cuong B. H. - Flavonoid and lignan compounds isolated from underground parts of the plant Podophylum tonkinense Gagnep. collected in Sapa, Lao Cai. Vietnam Pharm. J., **507** (2018) 48-53.
21. Walker M. B., Kimmel C. B. - A two-color acid-free cartilage and bone stain for zebrafish larvae. Biotech. Histochem., **82** (2007) 23-28. https://doi.org/10.1080/10520290701333558.

22. Chaturvedi P., Binhui Z., Zimmerman D. L., Belmont A. S. - Stable and reproducible transgene expression independent of proliferative or differentiated state using BAC TG-EMBED. Gene Ther., **25** (2018) 376-391. https://doi.org/10.1038/s41434-018-0021-z.
23. Hyun H. H., Park H., Jeong J., Kim J., Kim H., Oh H. I., Hwang H. S., Kim H. H. - Effects of watercress containing rutin and rutin alone on the proliferation and osteogenic differentiation of human osteoblast-like MG-63 cells. Korean J. Physiol. Pharmacol., **18** (2014) 347-352. https://doi.org/10.4196/kjpp.2014.18.4.347.
24. Hosseinzadeh H., Nassiri-Asl M. - Review of the protective effects of rutin on the metabolic function as an important dietary flavonoid. J. Endocrinol. Investig., **37** (2014) 783-788. https://doi.org/10.1007/s40618-014-0096-3.
25. Srivastava S., Bankar R., Roy P. - Assessment of the role of flavonoids for inducing osteoblast differentiation in isolated mouse bone marrow derived mesenchymal stem cells. Phytomedicine, **20** (2013) 683-690. https://doi.org/10.1016/j.phymed.2013.03.001.
26. Pickrell J. A. - Chapter 28 - Radiation and Health Effects. In *Handbook of Toxicology of Chemical Warfare Agents*, Academic Press (2009) 381-392.
27. de Witte W. E. A., Danhof M., van der Graaf P. H. - The implications of target saturation for the use of drug-target residence time. Nat. Rev. Drug Discov., **18** (2019) 84. https://doi.org/10.1038/nrd.2018.234.
28. To T. T., Witten P. E., Huysseune A., Winkler C. - An adult osteopetrosis model in medaka reveals the importance of osteoclast function for bone remodeling in teleost fish. Comp. Biochem. Physiol. C, **178** (2015) 68-75. https://doi.org/10.1016/j.cbpc.2015.08.007.
29. Renn J., Buttner A., To T. T., Chan S. J. H., Winkler C. - A col10a1:nlGFP transgenic line displays putative osteoblast precursors at the medaka notochordal sheath prior to mineralization. Dev. Biol., **381** (2013) 134-143. https://doi.org/10.1016/j.ydbio.2013.05.030.
30. Gera S., Pooladanda V., Godugu C., Swamy-Challa V., Wankar J., Dodoala S., Sampathi S. - Rutin nanosuspension for potential management of osteoporosis: effect of particle size reduction on oral bioavailability, in vitro and in vivo activity. Pharm. Dev. Technol., **25** (2020) 971-988. https://doi.org/10.1080/10837450.2020.1765378.
31. Morikane D., Zang L., Nishimura N. - Evaluation of the percutaneous absorption of drug molecules in zebrafish. Molecules, **25** (2020) 3974. https://doi.org/10.3390/molecules25173974.
32. Zhang F., Qin W., Zhang P., Hu Q. - Antibiotic toxicity and absorption in zebrafish using liquid chromatography-tandem mass spectrometry. PLoS One, **10** (2015) e0124805. https://doi.org/10.1371/journal.pone.0124805.
33. Yang C. Y., Hsiu S. L., Wen K. C., Lin S. P., Tsai S. Y., Hou Y. C., Chao P. D. L. - Bioavailability and metabolic pharmacokinetics of rutin and quercetin in rats. J. Food Drug Anal., **13** (2005) https://doi.org/10.38212/2224-6614.2517.