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Original Article

Flavonoids from *Dalbergia cochinchinensis*: Impact on osteoclastogenesis

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KEYWORDS
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Abstract *Background/purpose: Dalbergia cochinchinensi* has been widely used in traditional medicine because of its flavonoids. This study examined which components in *D. cochinchinensis* were capable of reducing or even stimulating the formation of bone-resorbing osteoclasts.
Materials and methods: We have isolated subfamilies of chalcones (isoliquiritigenin, butein), flavones (7-hydroxy-6-methoxyflavone) and neoflavanoids (5-methoxylatifolin), and performed an in vitro bioassay on osteoclastogenesis. The flavonoids were tested for their potential to change the expression of tartrate-resistant acid phosphatase (TRAP) and cathepsin K (CTSK) in murine bone marrow cultures being exposed to RANKL, M-CSF and TGF-β1 using RT-PCR, histochemistry and immunoassay.
Results: We could confirm that isoliquiritigenin and butein significantly lower the expression of TRAP and CTSK in this setting. Moreover, histochemistry supported the decrease of TRAP by the chalcones. We further observed a trend towards an increase of osteoclastogenesis in the presence of 5-methoxylatifolin and 7-hydroxy-6-methoxyflavone, particular in bone marrow cultures being exposed to RANKL and M-CSF. Consistently, the anti-inflammatory activity was restricted to isoliquiritigenin and butein in murine RAW 264.7 inflammatory macrophages stimulated by lipopolysaccharide (LPS). With respect to osteoblastogenesis, neither of the flavonoids but butyrate, a short chain fatty acid, increased the osteogenic differentiation marker alkaline phosphatase activity in ST2 murine mesenchymal cells.

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Conclusion: We have identified two flavonoids from *D. cochinchinensis* with a potential pro-osteoclastogenic activity and confirm the anti-osteoclastogenic activity of isoliquiritigenin and butein.

Introduction

Bone homeostasis is based on the balanced action of two antagonistic cells, the bone-resorbing osteoclasts and the bone-forming osteoblasts. Considering that the action of the osteoclasts and the osteoblasts are functionally coupled,[1] there is a great demand to understand how the coupling works and from a pharmacological standpoint, how it can be manipulated.[2] It is particular important in metabolic bone disease such as postmenopausal but also age- or cortisone-related osteoporosis to regain a balanced bone turnover.[2,3] Today's first-line osteoporosis therapies, apart from supplementing calcium and vitamin D, are either antiresorptive drugs targeting the osteoclast or anticatabolic drugs mainly supporting bone formation.[2] Examples are the bisphosphonates[4] and teriparatide,[5] respectively. However, there is great interest in natural plant-derived products to combat the catabolic changes of osteoporotic bone loss and potentially also inflammatory osteolysis associated with periodontitis, inflammatory bowel disease, and rheumatoid arthritis.

Flavonoid polyphenols including quercetin, rutin, luteolin, kaempferol, naringin are considered beneficial for the prevention and treatment of osteoporosis.[6] Although flavonoids comprise many compounds that differ in structure, their bone protective effects in postmenopausal women or in ovariectomized osteoporotic animals are quite similar. Most of the flavonoids increase bone mineral density and bone strength through enhancement of osteoblastogenesis and decrease in osteoclastogenesis, as well as increase in vascularization at fracture sites.[7—9] Moreover, non-flavonoid polyphenols are of possible use for prevention and treatment of osteoporosis.[10] This knowledge is mainly based on the capacity of the flavonoids to protect ovariectomized rodents from bone loss but also clinical research has been performed.[9] Overall these data support the relevance of flavonoids in the context of prevention and treatment of osteoporotic bone loss and presumably inflammatory osteolysis.

Dalbergia belonging to the family of *Fabaceae* are valued for their decorative and fragrant wood but are protected under the Convention on International Trade in Endangered Species of Wild Fauna and Flora. For research purpose, heartwood extracts and isolated components from the *Dalbergia sissoo* support facture healing and prevented bone loss in ovariectomized rats[11] and mice.[12,13] Consistently, 6,7,4′-trihydroxyflavanonem, 9-hydroxy-6,7-dimethoxydalbergiquinol, and 6,4′-dihydroxy-7-methoxyflavanone from *Dalbergia odorifera* inhibit in vitro osteoclast formation.[14-16] *Dalbergia* species also contain isoliquiritigenin, a flavonoid with chalcone structure (4,2′,4′-trihydroxychalcone)[17] know to reduce in vitro osteoclastogenesis[18—23] and bone loss in ovariectomized rats.[18] The same is true for 2′,3,4,4′-tetrahydroxychalcone named butein.[24] 2,4,5-trimethoxyldalbergiquinol isolated from *D. odorifera* was further reported to support osteogenic differentiation.[25] Thus, *Dalbergia* species are rich in flavonoids with the capacity to reduce osteoclastogenesis. However, this does not rule out that *Dalbergia* species contain flavonoids with the capacity to increase osteoclastogenesis.

Therefore, in this study, we tried to examine whether the isoliquiritigenin (IL) and butein (BU) contained in *D. cochinchinensis* could reduce osteoclastogenesis in vitro and whether the *D. cochinchinensis* compounds 7-hydroxy-6-methoxyflavone (HMF) and neoflavanoid 5-methoxylatifolin (ML) could increase osteoclastogenesis in vitro.

Materials and methods

Extraction of compounds

The isolation of the compounds was reported in detail elsewhere.[26] In brief, the dried sliced heartwood of *D. cochinchinensis* (20 kg) was extracted with 70% EtOH (reflux, 2 h, three times) (Xilong Scientific, Guangzhou, China). After the removal of EtOH (Xilong Scientific) under reduced pressure, the extract was suspended in water and partitioned sequentially with petroleum ether (60—90 °C) (Xilong Scientific), chloroform (Xilong Scientific), EtOAc (Xilong Scientific), and n-BuOH (Xilong Scientific), respectively.

The petroleum ether extract (381.2 g) was separated intonine fractions (Frs. 1—9) by applying silica gel column (Qingdao Haiyang Chemical, Qingdao, China), eluted with petroleum ether-EtOAc (50:1 to 1:1). Fr. 7 (6.0 g) was separated into ten fractions (Frs. 7 A-7J) by applying silica gel column (Qingdao Haiyang Chemical), eluted with petroleum ether-CH_2Cl_2(Xilong Scientific) (2:1 to 1:2). Fr. 7H (160.0 mg) was purified by Sephadex LH-20 (Pharmacia-Fine Chemical, Uppsala, Sweden) with CH_2Cl_2—MeOH (Xilong Scientific) (1:1) to give 5-methoxylatifolin (ML; 82.2 mg). The chemical structure of ML was confirmed by nuclear magnetic resonance spectroscopy.[27] ML was dissolved in dimethyl sulfoxide (Sigma—Aldrich, St Louis, MO, USA) and stored in aliquots until being used for the in vitro testing.

The chloroform extract (902.6 g) was separated into 9 fractions (Frs. 1—9) by silica gel column (Qingdao Haiyang Chemical) and eluted with gradient mixture of petroleum ether-EtOAc (50:1 to 1:1). Fr. 4 was subjected to silica gel (Qingdao Haiyang Chemical) using CH_2Cl_2—MeOH (100:1 to

1:1), followed by Sephadex LH-20 (PharmaciaFine Chemical) with CH_2Cl_2—MeOH (1:1) to give compound 7-hydroxy-6-methoxyflavone (HMF) (421.5 mg). The chemical structures of HMF was confirmed by nuclear magnetic resonance spectroscopy.[28] HMF was dissolved in dimethyl sulfoxide (Sigma—Aldrich) and stored in aliquots until being used for the in vitro testing.

The EtOAc extract (1397.7 g) was separated into 23 fractions (Fr. 1—23) by the silica gel column (Qingdao Haiyang Chemical) using CH_2Cl_2—MeOH (100:0 to 1:1) as eluent. Fr. 16 was separated over silica gel (Qingdao Haiyang Chemical), eluted with CH_2Cl_2—MeOH (50:1 to 1:1), next octadecylsilyl silica gel (Thermo Fisher Scientific, Waltham, MA, USA) using 65% MeOH (Xilong Scientific) as an eluent to obtain compound isoliquiritigenin (IL; 90.4 mg) and butein (BU; 6.9 mg). The chemical structures of IL and BU were confirmed by nuclear magnetic resonance spectroscopy.[29] They were dissolved in dimethyl sulfoxide (Sigma—Aldrich) and stored in aliquots until being used for the in vitro testing.

Osteoclast culture

BALB/c mice at the age of 6—8 weeks were purchased from Animal Research Laboratories, Himberg, Austria. Bone marrow cells were collected from the femora and tibiae. Bone marrow cells were seeded at 1×10^6 cells/cm^2 into 24-well plates (VWR International, Radnor, PA, USA) and grown for 10 days in alpha Modified Eagle Medium (αMEM) (Invitrogen, Grand Island, NY, USA) supplemented with 10% fetal calf serum (FCS) (Bio&Sell GmbH, Nuremberg, Germany) and 1% of 10,000 units penicillin (Sigma—Aldrich) and 10 mg streptomycin/ml (Sigma—Aldrich). We further added 20 ng/ml mouse macrophage colony-stimulating factor (M-CSF) (ProSpec-Tany TechnoGene, Rehovot, Israel) and 20 ng/ml receptor activator of nuclear factor kappa-B ligand (RANKL) (ProSpec, Ness-Ziona, Israel) either with or without 10 ng/mL TGF-β1 (ProSpec, Ness-Ziona, Israel) for 10 days.[30] At the same time 20 μM of IL, BU, HMF, and ML were added to the culture medium. Histochemical staining for TRAP was done following the instruction of the manufacturer (Sigma-Aldrich). In addition, the expression of TRAP and CTSK was analyzed.

Macrophage culture

RAW 264.7 macrophage-like cells (ATCC; LGC Standards GmbH, Wesel, Germany) grown in serum-containing DMEM (Invitrogen) were seeded at 1×10^6/cm^2 for the experiments. RAW264.7 cells were exposed to 20 μM of IL, BU, HMF, and ML for 30 min before adding 100 ng/ml lipopolysaccharide (LPS) (Sigma—Aldrich) from *Escherichia coli* 0111: B41 (Sigma—Aldrich) for 24 h. The expression of IL6 and CXCL2 was evaluated by RT-PCR (Bio-Rad Laboratories, Hercules,CA, USA).

Mesenchymal cell culture

ST2 murine bone marrow stromal cells (RIKEN Cell Bank, Tsukuba, Japan) were expanded in serum-containing DMEM (Sigma—Aldrich) and seeded at 3×10^5/cm^2 for the experiments. ST2 cells were exposed to 20 μM of IL, BU, HMF, and ML or 10 μM butyrate (Sigma—Aldrich) for 7 days. Butyrate served as a positive control for increasing alkaline phosphatase activity.[31] For histochemical staining of alkaline phosphatase, fixed cells were incubated with a substrate solution containing naphthol AS-TR phosphate (Sigma—Aldrich) and fast blue BB salt (Sigma-Aldrich).[32] Images were captured under a light microscope (Echor-evolve microscope; Euromex, Arnheim, Netherlands).

RT-PCR and immunoassay

Total RNA was isolated with the ExtractMe total RNA kit (Blirt S.A., Gdańsk, Poland). Complementary DNA was synthesized through reverse transcription of the total RNA (LabQ, Labconsulting, Vienna, Austria) and polymerase chain reaction was performed with a master mix from LabQ (Labconsulting). Amplification was monitored on the CFX Connect™ Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA, USA). Primer sequences are provided in Table 1. The mRNA levels were calculated by normalization to the housekeeping gene GAPDH (R&D Systems) using the $\Delta\Delta$Ct method.

Statistical analysis

All experiments were repeated at least three times. Data from individual experiments are shown as bar graphs with standard deviation. Data are described as x-fold change compared to unstimulated control. Statistical analysis was based on repeated measures one-way ANOVA with Greenhouse-Geisser correction and Dunnett's multiple comparison test. The *P*-values are indicated compared to the stimulated group but without the flavonoids. Significance was set at $P < 0.05$. Data were analyzed by the Prism 8.0e software (GraphPad Software, San Diego, CA, USA).

Results

Isolation of flavonoids from *D. cochinchinensis*

To isolate chemically defined natural compounds we reconstituted the ethanolic extracts of the heartwood of *D. cochinchinensis* with petroleum ether, chloromethane, ethyl acetate, and n-butanol dichloromethane and ethyl acetate. The petroleum ether extract was used to isolate ML (Fig. 1). Chloroform extract allowed HMF to be isolated (Fig. 1). The ethyl acetate extract was the source of IL and BU (Fig. 1).

Isoliquiritigenin (IL) and butein (BU) but 7-hydroxy-6-methoxyflavone (HMF) and 5-methoxylatifolin (ML) decrease the formation of osteoclasts

To understand how the flavonoids extracted from the heartwood of *D. cochinchinensis* affect osteoclastogenesis, we exposed murine bone marrow cells to the flavonoids in the presence of M-CSF, RANKL and TGF-β1. In the presence of IL and BU, the process of osteoclastogenesis was

Table 1 The primer sequences. TRAP:tartrate-resistant acid phosphatase; CTSK:cathepsin K; IL6:interleukin 6; CXCL2:C-X-C motif ligand 2; GAPDH:glyceraldehyde-3-phosphate dehydrogenase.

Gene	Sequence (5'→3')	Sequence (3'→5')
TRAP	Forward: CGTCTCTGCACAGATTGCAT	Reverse: AAGCGCAAACGGTAGTAAGG
CTSK	Forward: TGTATAACGCCACGGCAAA	Reverse: GGTTCACATTATCACGGTCACA
IL6	Forward: GCTACCAAACTGGATATAATCAGGA	Reverse: CCAGGTAGCTATGGTACTCCAGAA
CXCL2	Forward: CATCCAGAGCTTGAGTGTGACG	Reverse: GGCTTCAGGGTCAAGGCAAAC
GAPDH	Forward: AACTTTGGCATTGTGGAAGG	Reverse: GGATGCAGGGATGATGTTCT

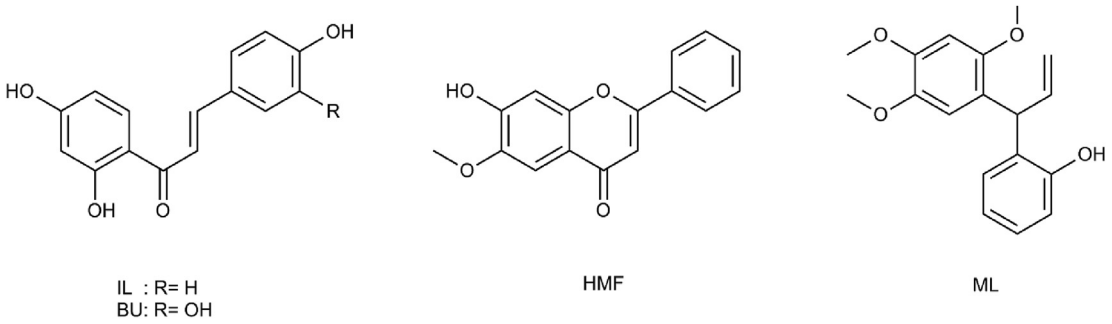

Figure 1 The structures of flavonoids. The ethanolic extract of the heartwood of *D. cochinchinensis* was further separated allowing the isolation of isoliquiritigenin (IL), butein (BU), 7-hydroxy-6-methoxyflavone (HMF), and 5-methoxylatifolin (ML).

significantly reduced as indicted by the reduction of TRAP positive cells and the respective expression changed of TRAP and CTSK. On the other hand, there was a trend towards an increased osteoclastogenesis in the presence of HMF and ML (Figs. 2 and 3). The increase in osteoclastogenesis was even more obvious when the bone marrow cells were cultivated with M-CSF and RANKL alone (Figs. 4 and 5). These findings confirm that the IL and BU are potent in suppressing osteoclastogenesis and further suggest that *D. cochinchinensis* contains flavonoids with the capacity to stimulate osteoclastogenesis in vitro.

Flavonoids lower the lipopolysaccharide (LPS)-induced inflammatory response of RAW 264.7 macrophages

To further confirm their biological activity, we have exposed RAW 264.7 to the four flavonoids before provoking an inflammatory response with LPS.[33,34] We found that the strong LPS-induced increase of IL6 and CXCL2 expression was considerably reduced in the presence of 20 μM IL, BU,

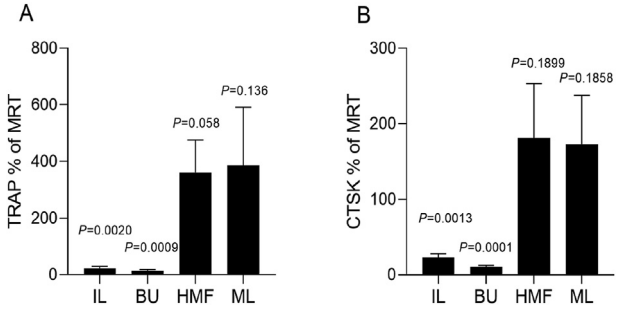

Figure 3 IL and BU but not HMF and ML suppress TRAP and CTSK expression in osteoclast cells. Osteoclast cells were exposed to 20 μM of isoliquiritigenin (IL), butein (BU), 7-hydroxy-6-methoxyflavone (HMF), and 5-methoxylatifolin (ML) and treated with MCSF and RANKL for 10 days. MR stands for MCSF and RANKL. Data show the % changes in tartrate-resistant acid phosphatase (TRAP) and cathepsin K (CTSK) expression compared to cells exposed to MR only. Significance based on a RM one-way ANOVA with the Greenhouse-Geisser correction, n = 3.

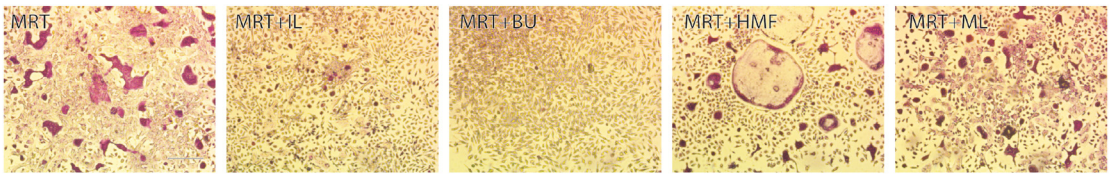

Figure 2 Morphology of osteoclasts cells exposed to IL, BU, HMF, and ML. Osteoclasts cells were exposed to 20 μM of isoliquiritigenin (IL), butein (BU), 7-hydroxy-6-methoxyflavone (HMF), and 5-methoxylatifolin (ML) and treated with MCSF, RANKL and TGF-β1 (MRT) for 10 days. MRT indicates control without flavonoids. Images indicate IL and BU but not HMF and ML caused a decrease in osteoclastogenesis. The size bar represents 200 μm.

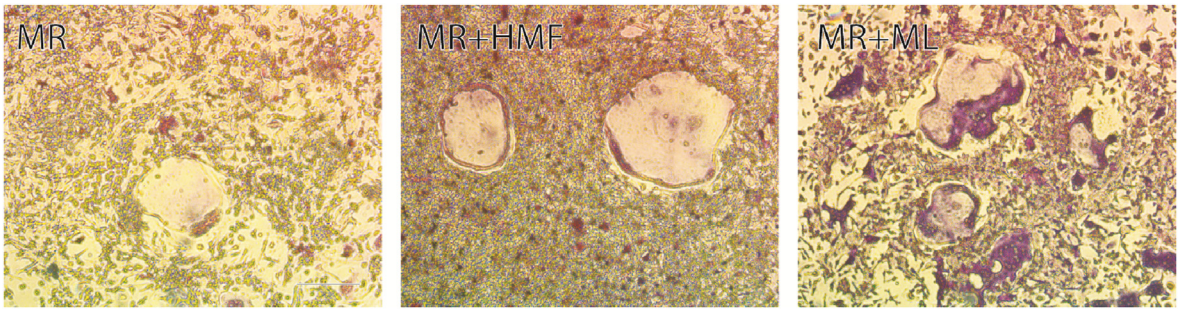

Figure 4 Morphology of osteoclast-like cells exposed to flavonoids. Murine bone marrow cells were exposed to 20 μM of 7-hydroxy-6-methoxyflavone (HMF), and 5-methoxylatifolin (ML) and treated with MCSF and RANKL for 10 days. MR stands for MCSF and RANKL. The images indicate HMF and particular ML to stimulate osteoclastogenesis in vitro. The size bar represents 200 μm.

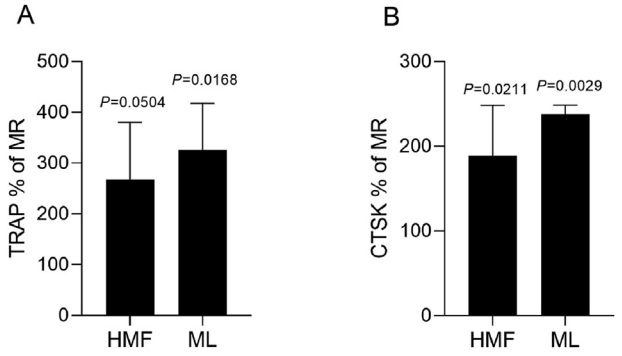

Figure 5 HMF and ML increased TRAP and CTSK expression in osteoclast cells. Osteoclast cells were exposed to 20 μM of 7-hydroxy-6-methoxyflavone (HMF) and 5-methoxylatifolin (ML) and treated with MCSF and RANKL for 10 days. Data show the % changes in tartrate-resistant acid phosphatase (TRAP) and cathepsin K (CTSK) expression compared to unstimulated cells. Significance was based on an ordinary one-way ANOVA, n = 3.

HMF, and ML in RAW 264.7 cells (Fig. 6). Overall, BU was most potent to reduce the inflammatory response of macrophages to LPS.

Flavonoids had no impact on osteogenic differentiation of ST2 murine mesenchymal cells

To identify a possible modulation of the osteogenic differentiation, we exposed ST2 murine mesenchymal cells to the four flavonoids and butyrate serving as a positive control. As expected,[31] butyrate caused ST2 cells to increase the expression of alkaline phosphatase activity as indicated by the blue staining. There was however no obvious change in the staining intensity of alkaline phosphatase when ST2 cells were exposed to either of the four flavonoids (Fig. 7). We have performed gene expression analysis and also observed no obvious change in the expression of alkaline phosphatase and osteopontin (data not shown). There data suggest that even though the flavonoids are capable to modulate osteoclastogenesis and reduce the LPS response of macrophage, the osteogenic differentiation of murine mesenchymal cell remains unaffected.

Discussion

The grace of old age stands against the age-related diseases affecting the musculoskeletal and dental apparatus, with osteoporosis and the associated high fracture risk.[35]

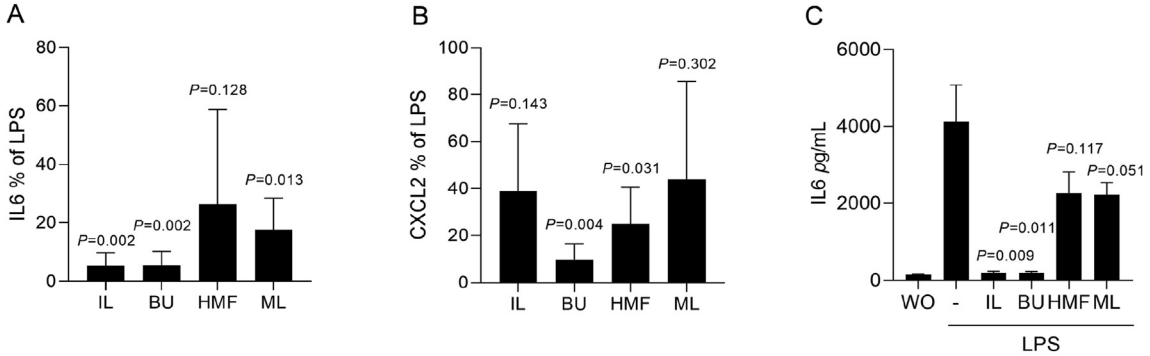

Figure 6 Flavonoids suppress LPS-induced expression of cytokines in RAW 264.7 cells. RAW 264.7 cells were exposed to 20 μM of isoliquiritigenin (IL), butein (BU), 7-hydroxy-6-methoxyflavone (HMF), and 5-methoxylatifolin (ML) for 30 min before cells were treated with 100 ng/ml lipopolysaccharide (LPS) for 12 h. Data show the % changes in (A) Interleukin 6 (IL6), and (B) C-X-C motif ligand 2 (CXCL2) expression compared to 100% expression of LPS-exposed cells. The immunoassay shows the release of (C) IL6 into the supernatant of the respective cultures. Significance was set at $P < 0.05$, n = 3.

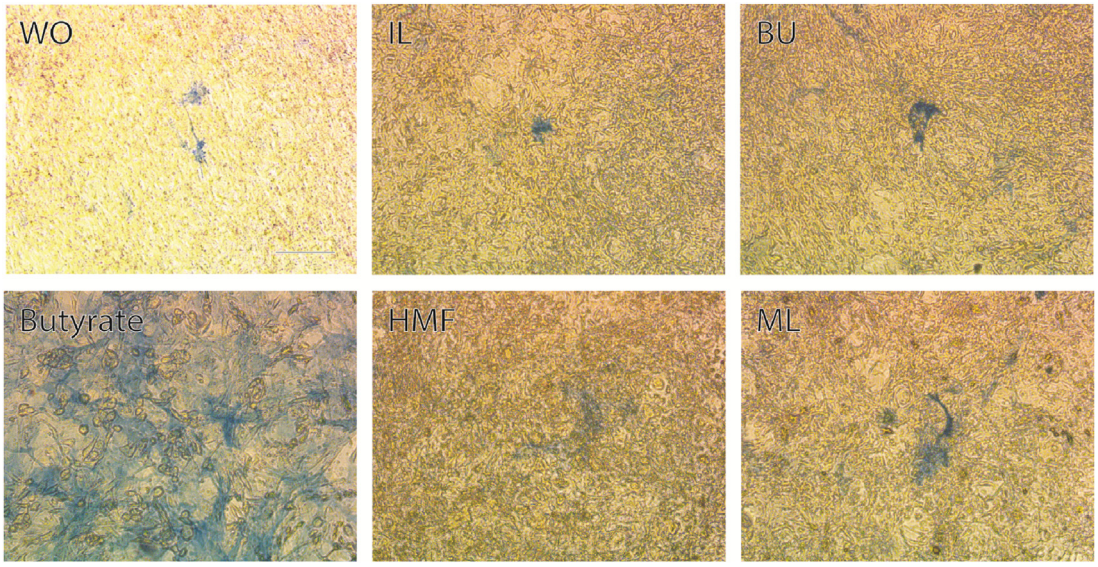

Figure 7 Morphology of ST2 cells exposed to IL, BU, HMF, and ML. ST2 cells were exposed to 20 μM of isoliquiritigenin (IL), butein (BU), 7-hydroxy-6-methoxyflavone (HMF), and 5-methoxylatifolin (ML) and 10 μM butyrate for 7 days. Without (WO) indicates unstimulated control. The phase-contrast images indicate IL, BU, HMF, and ML caused no change in the appearance of cells staining positive for alkaline phosphatase activity. The size bar represents 100 μm.

Patients have benefitted from the understanding of how pharmacological drugs can modulate the imbalanced remodeling process where osteoclastic bone resorption exceeds osteoblastic bone formation. Apart from the well-established pharmacological drugs and their approved indication for lowering fracture risk,[4,5] there is great interest in natural plant-derived products to possible prevent or even treat osteoporosis.[9,10] Plant-derived products such as IL and BU may also diminish the inflammatory osteolysis associated with colitis[36,37] and arthritis.[23,38] Inspired by this concept, we have used *D. cochinchinensis* to isolate its flavonoids. The main finding of the present research was that we could confirm that IL and BU are potent in reducing osteoclastogenesis in vitro. Unexpected and therefore worth to be reported was our other observation that HMF and ML exert a modest activity in increasing osteoclastogenesis.

If we relate our findings to those of others we have to refer to the elegant work showing that *Dalbergia* species support facture healing and prevented bone loss in ovariectomized rats[11] and mice.[12,13] This beneficial effect can be explained by *Dalbergia* species containing IL and BU,[17] both compounds know to reduce in vitro osteoclastogenesis.[19–21,24] Also, other compounds from *Dalbergia* species such as 6,7,4′-trihydroxyflavanone, 9-hydroxy-6,7-dimethoxydalbergiquinol, and 6,4′-dihydroxy-7-methoxyflavanone inhibit osteoclast formation.[14–16] Thus, our findings are in great support of what is known from IL and BU to reduce and even suppress in vitro osteoclastogenesis. However, we observed that HMF and ML can at least moderately increase osteoclastogenesis; this was a rather unexpected finding we cannot relate to others. Recent reviews suggest that there are several molecules involved in the effect of flavonoids on osteoporotic bone, hence, the existence of flavonoids with the capacity to stimulate osteoclastogenesis should not be ruled out.[8]

The impressive impact of IL and BU to reduce osteoclastogenesis, however, does not necessarily mean that IL and BU stimulate osteoblastogenesis. We have performed a screening of the flavonoids aiming to see an increase in the alkaline phosphatase activity taking advantage of the murine bone marrow stromal cell line ST2.[39] Butyrate served as positive control knowing that blocking histone deacetylase increases osteogenic differentiation as expressed by alkaline phosphatase activity.[31] Neither IL nor BU, but also not HMF and ML caused a visible increase in osteogenic differentiation; hence, we have not further gone into details. However, it should not be ruled out that other flavonoids modulate alkaline phosphatase activity as for instance, 2,4,5-trimethoxyldalbergiquinol isolated from *D. odorifera* increased osteogenic differentiation.[25] Also, quercetin and rutin[40] as well as *Tridax procumbens* flavonoids[41] increased alkaline phosphatase activity. Hence, there are probable other flavonoids from *Dalbergia* with an osteogenic potential.

The clinical relevance of our findings remains speculative but accumulating evidence suggesting that *Dalbergia* species prevented bone loss in ovariectomized rats[11] and mice[12,13] implicates a therapeutic relevance to possible prevent bone loss and once osteoporosis occurs, helping to regain bone strength by fine tuning of the remodeling balance towards anabolism. It can further be speculated that flavonoids isolated from *Dalbergia* species including IL and BU may be used for coating of dental implants to reduce bone loss following implant installation[42] or to supplement bone grafts with flavonoids preventing their resorption at augmentation sites.[43] Interesting will be to determine if IL and BU are capable of resolving an inflammatory reaction and thereby help to compensate inflammatory osteolysis and tissue destruction.[44]

Our pilot study has limitation, for instance, we have not evaluated if the flavonoids are capable to reduce the

resorptive activity of mature osteoclasts, apart from reducing their differentiation. Another open question remains the molecular mechanism how HMF and ML exert their weak pro-osteoclastogenic activity. Heme oxygenase-1 (HO-1; encoded by Hmox1), a downstream target of the Nrf2 transcription factor, has been postulated to be a negative regulator of osteoclasts differentiation[45] and IL pushes HO-1 expression in macrophages.[46] Thus, the anti-osteoclastogenic similar to the anti-inflammatory activity is likely mediated via this pathway. Future studies should evaluate if HMF and ML possible increase HO-1-Nrf2 signaling making the hematopoietic cells more susceptive to RANKL signaling.[47] Considering that flavonoids are capable to induce HO-1 signaling and knowing that upregulation of HO-1 inhibits the maturation and mineralization of osteoblasts,[48] our findings that none of the flavonoids stimulated alkaline phosphatase activity is not surprising. Future studies should evaluate if our flavonoids can hinder butyrate from exerting its strong increase in alkaline phosphatase activity.[31] Future research should also consider possible dose- and time-response effect since biphasic regulations can not be ruled out. Moreover, preclinical studies are required to prove the bioavailability of the compounds.

Taken together our findings add to the accumulating evidence that *D. cochinchinensis* derived isoliquiritigenin and butein have a potent anti-osteoclastogenic and anti-inflammatory potential in hematopoietic cells but no visible impact on alkaline phosphatase activity in mesenchymal cell. 7-hydroxy-6-methoxyflavone and 5-methoxylatifolin also exert an anti-inflammatory activity but has a moderate activity to stimulate osteoclastogenesis. These finding inspire research to understand how the molecular structure of chalcones targets molecules involved in osteoclastogenesis and macrophage activation.

Declaration of competing interest

The authors declare that they have no conflict of interest.

Acknowledgments

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References

1. Sims NA, Martin TJ. Osteoclasts provide coupling signals to osteoblast lineage cells through multiple mechanisms. *Annu Rev Physiol* 2020;82:507—29.
2. Rachner TD, Khosla S, Hofbauer LC. Osteoporosis: now and the future. *Lancet* 2011;377:1276—87.
3. Seeman E, Delmas PD. Bone quality–the material and structural basis of bone strength and fragility. *N Engl J Med* 2006;354:2250—61.
4. Ebetino FH, Sun S, Cherian P, et al. Bisphosphonates: the role of chemistry in understanding their biological actions and structure-activity relationships, and new directions for their therapeutic use. *Bone* 2022;156:116289.
5. Neer RM, Arnaud CD, Zanchetta JR, et al. Effect of parathyroid hormone (1-34) on fractures and bone mineral density in postmenopausal women with osteoporosis. *N Engl J Med* 2001;344:1434—41.
6. 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* 2022;14:523.
7. Ramesh P, Jagadeesan R, Sekaran S, Dhanasekaran A, Vimalraj S. Flavonoids: classification, function, and molecular mechanisms involved in bone remodelling. *Front Endocrinol* 2021;12:779638.
8. Rodriguez V, Rivoira M, Picotto G, de Barboza GD, Collin A, Tolosa de Talamoni N. Analysis of the molecular mechanisms by flavonoids with potential use for osteoporosis prevention or therapy. *Curr Med Chem* 2021:2913—36.
9. Bellavia D, Dimarco E, Costa V, et al. Flavonoids in bone erosive diseases: perspectives in osteoporosis treatment. *Trends Endocrinol Metab* 2021;32:76—94.
10. Bellavia D, Caradonna F, Dimarco E, et al. Non-flavonoid polyphenols in osteoporosis: preclinical evidence. *Trends Endocrinol Metab* 2021;32:515—29.
11. Karvande A, Khedgikar V, Kushwaha P, et al. Heartwood extract from *Dalbergia sissoo* promotes fracture healing and its application in ovariectomy-induced osteoporotic rats. *J Pharm Pharmacol* 2017;69:1381—97.
12. Choudhary D, Kushwaha P, Gautam J, et al. Fast and long acting neoflavonoids dalbergin isolated from *Dalbergia sissoo* heartwood is osteoprotective in ovariectomized model of osteoporosis: osteoprotective effect of *Dalbergin*. *Biomed Pharmacother* 2016;83:942—57.
13. Gautam J, Kumar P, Kushwaha P, et al. Neoflavonoid dalbergiphenol from heartwood of *Dalbergia sissoo* acts as bone savior in an estrogen withdrawal model for osteoporosis. *Menopause* 2015;22:1246—55.
14. Kim EN, Kim YG, Lee JH, Min BS, Jeong GS. 6,7,4'-Trihydroxyflavone inhibits osteoclast formation and bone resorption in vitro and in vivo. *Phytother Res* 2019;33:2948—59.
15. Kim JY, Kim JY, Cheon YH, et al. 9-Hydroxy-6,7-dimethoxydalbergiquinol inhibits osteoclast differentiation through down-regulation of Akt, c-Fos and NFATc1. *Int Immunopharm* 2014;20:213—20.
16. Im NK, Choi JY, Oh H, Kim YC, Jeong GS. 6,4'-Dihydroxy-7-methoxyflavanone inhibits osteoclast differentiation and function. *Biol Pharm Bull* 2013;36:796—801.
17. Chan SC, Chang YS, Wang JP, Chen SC, Kuo SC. Three new flavonoids and antiallergic, anti-inflammatory constituents from the heartwood of *Dalbergia odorifera*. *Planta Med* 1998;64:153—8.
18. Joyce KM, Wong CP, Scriven IA, et al. Isoliquiritigenin decreases bone resorption and osteoclast differentiation. *Mol Nutr Food Res* 2022;66:e2100974.
19. Jeong S, Lee S, Kim K, et al. Isoliquiritigenin derivatives inhibit RANKL-induced osteoclastogenesis by regulating p38 and NF-kappaB activation in RAW 264.7 Cells. *Molecules* 2020;25:3908.
20. Liu S, Zhu L, Zhang J, Yu J, Cheng X, Peng B. Anti-osteoclastogenic activity of isoliquiritigenin via inhibition of NF-kappaB-dependent autophagic pathway. *Biochem Pharmacol* 2016;106:82—93.
21. Zhu L, Wei H, Wu Y, et al. Licorice isoliquiritigenin suppresses RANKL-induced osteoclastogenesis in vitro and prevents inflammatory bone loss in vivo. *Int J Biochem Cell Biol* 2012;44:1139—52.

22. Carnovali M, Banfi G, Mariotti M. Liquiritigenin reduces osteoclast activity in zebrafish model of glucocorticoid-induced osteoporosis. *J Pharmacol Sci* 2020;143:300—6.
23. Ji B, Zhang Z, Guo W, et al. Isoliquiritigenin blunts osteoarthritis by inhibition of bone resorption and angiogenesis in subchondral bone. *Sci Rep* 2018;8:1721.
24. Sung B, Cho SG, Liu M, Aggarwal BB. Butein, a tetrahydroxychalcone, suppresses cancer-induced osteoclastogenesis through inhibition of receptor activator of nuclear factor-kappaB ligand signaling. *Int J Cancer* 2011;129:2062—72.
25. Yun HM, Park KR, Quang TH, et al. 2,4,5-Trimethoxyldalbergiquinol promotes osteoblastic differentiation and mineralization via the BMP and Wnt/beta-catenin pathway. *Cell Death Dis* 2015;6:e1819.
26. Liu Rh, Wen XC, Shao F, Zhang PZ, Huang HL, Zhang S. Flavonoids from heartwood of *Dalbergia cochinchinensis*. *Chinese Herbal Medicines* 2016;8:89—93 [In Chinese, English abstract].
27. Nuanta Muangnoicharoen AWF. Neoflavanoids of *Dalbergia parviflora*. *Phytochemistry* 1982;21:767—72.
28. Pathak V, Shirota O, Sekita S, et al. Antiandrogenic phenolic constituents from *Dalbergia cochinchinensis*. *Phytochemistry* 1997;46:1219—23.
29. Yang H, Wang D, Tong L, Cai BC. Flavonoid aglycones of *Oxytropis falcata*. *Chem Nat Compd* 2009;45:239—41.
30. Fuller K, Lean JM, Bayley KE, Wani MR, Chambers TJ. A role for TGFbeta(1) in osteoclast differentiation and survival. *J Cell Sci* 2000;113(Pt 13):2445—53.
31. Schroeder TM, Westendorf JJ. Histone deacetylase inhibitors promote osteoblast maturation. *J Bone Miner Res* 2005;20:2254—63.
32. Gruber R, Kandler B, Fischer MB, Watzek G. Osteogenic differentiation induced by bone morphogenetic proteins can be suppressed by platelet-released supernatant in vitro. *Clin Oral Implants Res* 2006;17:188—93.
33. Panahipour L, Kochergina E, Laggner M, et al. Role for lipids secreted by irradiated peripheral blood mononuclear cells in inflammatory resolution in vitro. *Int J Mol Sci* 2020;21:4694.
34. Panahipour L, Kochergina E, Kreissl A, Haiden N, Gruber R. Milk modulates macrophage polarization in vitro. *Cytokine X* 2019;1:100009.
35. Black DM, Rosen CJ. Clinical practice. postmenopausal osteoporosis. *N Engl J Med* 2016;374:254—62.
36. Choi YH, Bae JK, Chae HS, et al. Isoliquiritigenin ameliorates dextran sulfate sodium-induced colitis through the inhibition of MAPK pathway. *Int Immunopharm* 2016;31:223—32.
37. Lee SD, Choe JW, Lee BJ, et al. Butein effects in colitis and interleukin-6/signal transducer and activator of transcription 3 expression. *World J Gastroenterol* 2015;21:465—74.
38. Zheng W, Zhang H, Jin Y, et al. Butein inhibits IL-1beta-induced inflammatory response in human osteoarthritis chondrocytes and slows the progression of osteoarthritis in mice. *Int Immunopharm* 2017;42:1—10.
39. Ogawa M, Nishikawa S, Ikuta K, et al. B cell ontogeny in murine embryo studied by a culture system with the monolayer of a stromal cell clone, ST2: B cell progenitor develops first in the embryonal body rather than in the yolk sac. *EMBO J* 1988;7:1337—43.
40. 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* 2013;20:683—90.
41. Al Mamun MA, Hosen MJ, Islam K, Khatun A, Alam MM, Al-Bari MA. Tridax procumbens flavonoids promote osteoblast differentiation and bone formation. *Biol Res* 2015;48:65.
42. Vasak C, Busenlechner D, Schwarze UY, et al. Early bone apposition to hydrophilic and hydrophobic titanium implant surfaces: a histologic and histomorphometric study in minipigs. *Clin Oral Implants Res* 2014;25:1378—85.
43. Saulacic N, Bosshardt DD, Jensen SS, Miron RJ, Gruber R, Buser D. Impact of bone graft harvesting techniques on bone formation and graft resorption: a histomorphometric study in the mandibles of minipigs. *Clin Oral Implants Res* 2015;26:383—91.
44. Schett G, Neurath MF. Resolution of chronic inflammatory disease: universal and tissue-specific concepts. *Nat Commun* 2018;9:3261.
45. Florczyk-Soluch U, Jozefczuk E, Stepniewski J, et al. Various roles of heme oxygenase-1 in response of bone marrow macrophages to RANKL and in the early stage of osteoclastogenesis. *Sci Rep* 2018;8:10797.
46. Lee SH, Kim JY, Seo GS, Kim YC, Sohn DH. Isoliquiritigenin, from *Dalbergia odorifera*, up-regulates anti-inflammatory heme oxygenase-1 expression in RAW264.7 macrophages. *Inflamm Res* 2009;58:257—62.
47. Tsukasaki M, Takayanagi H. Osteoimmunology: evolving concepts in bone-immune interactions in health and disease. *Nat Rev Immunol* 2019;19:626—42.
48. Lin TH, Tang CH, Hung SY, et al. Upregulation of heme oxygenase-1 inhibits the maturation and mineralization of osteoblasts. *J Cell Physiol* 2010;222:757—68.