

**Bone Anabolic Effect of Flavonoids from *Herba
Epimedii* in zebrafish and Medaka**

by

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**Institute of Chinese Medical Sciences
University of Macau**

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碩士學位論文

以斑马鱼及青鱈为体内模型研究中藥淫羊藿 黄酮类物质的促骨骼生成作用

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University of Macau

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摘 要

以斑马鱼及青鱗为体内模型研究中藥淫羊藿黄酮类物质的促骨骼生成作用

李振华

导师：李铭源

骨质疏松症是当今最为常见的骨骼疾病之一，其特点为骨矿物密度降低，骨折风险显著增加。一般而言，骨质疏松症的治疗药物可分为抗骨重吸收药物和促骨生成药物两大类。抗骨重吸收药物通过抑制破骨细胞活性达到降低骨重吸收作用的目的，虽然抗骨重吸收药物是目前治疗骨质疏松症的主要手段，但缺点是此类药物对于已有的骨折疗效不佳，而促骨生成药物具有增加骨量，改善骨质量，提高骨骼强度等功效成为现今新型抗骨质疏松药物的开发热点。

中药淫羊藿是多年生草本植物淫羊藿的地上部分，其中富含包含如淫羊藿苷、染料木黄酮等诸多活性成分。近年来研究发现淫羊藿黄酮类化合物不仅具有潜在的促骨生成活性，而且也有抑制破骨细胞、抗骨重吸收的能力。为进一步了解淫羊藿黄酮类化合物的药理活性，我们以斑马鱼和青鱗为体内模型分析上述黄酮类化合物对成骨细胞分化和基质矿化的影响。实验发现经过淫羊藿素、淫羊藿次苷、淫羊藿苷和朝藿定C等一系列具有相似结构的黄酮处理过的斑马鱼依次出现骨骼特异的绿色荧光现象，结果表明这种有先后次序出现荧光的现象很可能与此类黄酮苯环上的糖基代谢有关。斑马鱼的骨骼红色荧光染色实验显示淫羊藿素可显著抑制骨骼染色剂引起的红色荧光，这或许表明淫羊藿素与

染色剂一样都具有与钙离子结合能力，分光光度检测实验验证在碱性环境中淫羊藿素可与钙离子、血清蛋白反应而表现出绿色荧光现象。

实验发现转基因青鳉在淫羊藿苷给药处理后其成熟成骨细胞数目下降但在整体水平有促进骨生成的作用，这表明淫羊藿素及其衍生物可能促进成骨细胞矿化作用，或者抑制破骨细胞重吸收活动，或两者兼而有之，而以斑马鱼为模型的基因表达分析显示淫羊藿素可显著调节成骨细胞及软骨细胞的生理活性。此外另一黄酮——染料木黄酮在实验中表现出促骨生成作用效果。以往研究表明染料木黄酮具有促进大鼠和成骨细胞系生成骨骼的潜力，此次在斑马鱼模型实验中也得到相似结果。

综上所述，我们考察了斑马鱼和青鳉体内模型应用于药物体内代谢研究及促骨生成药物筛选的可行性，并发现淫羊藿中黄酮类成分淫羊藿素及其衍生物有调节成骨细胞的药理活性。

关键词：斑马鱼，青鳉，骨质疏松，淫羊藿，淫羊藿素，淫羊藿次苷，淫羊藿苷，朝藿定 C，骨钙素

ABSTRACT

Bone Anabolic Effect of Flavonoids from *Herba Epimedii* in zebrafish and Medaka

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Supervisor: Prof. Simon Ming-Yuan Lee

Osteoporosis is one of the most popular bone diseases in the world, which is marked by low bone mineral density and increased risk of bone fractures. Three main mechanisms leading to osteoporosis are inadequate peak bone mass, excessive bone resorption and inadequate bone formation. Though antiresorptive agents are the most commonly used medicine methods in osteoporosis treatment, the draw back is obvious that the drugs could not reverse the bone loss process. On the other hand, anabolic agents have the ability to increase bone mass, improve bone quality and increase bone strength, and anabolic agents are potential new therapeutic agents for antiosteoporosis therapy.

Herba Epimedii, which contains several pharmacologically active compounds such as icariin and epimedin C, has been widely used in China in the treatment of various diseases. Recent researches have revealed that compounds in *Herba Epimedii* not only have potential bone anabolic activity, but also have the capability to inhibit osteoclastic bone resorption by *in vitro* and *in vivo* models. In order to study the effect of these flavonoids on osteoblasts differentiation and matrix mineralization, we

employed zebrafish and medaka *in vivo* models. Treated zebrafish embryos with a variety of *Herba Epimedii* derived flavonoids showed bone-specific fluorescence. The bone-specific fluorescence was observed faster in icaritin treatment followed by icariside II, icariin and epimedin C. Icariside II, icariin and epimedin C are one-, two- and three-glycosides of icaritin and is normally metabolized *in vivo* to its aglycoside form. This result indicates that the bone-specific fluorescence may be due to this aglycoside metabolite. Combined incubation of zebrafish embryos in live alizarin red stain and icaritin showed that icaritin-induced bone-specific fluorescence was greatly reduced, indicating perhaps that these two compounds may compete for calcium binding.

Results obtained from *osteocalcin:gfp* transgenic medaka, which underwent the icariin treatment revealed overall positive effects in bone formation, suggests that icaritin and its derivatives probably modulate osteoblast mineralization, or inhibit osteoclast resorption. Furthermore, the anabolic effect of genistein was investigated. It was observed that genistein have bone anabolic potential, which is consistent with previous studies using other models.

In conclusion, zebrafish is a potential secondary metabolism model as well as *in vivo* model for bone anabolic chemical screening. Icaritin and its derivatives probably have bone anabolic potential

KEY WORDS: Zebrafish, medaka, osteoporosis, *Herba Epimedii*, epimedium, icaritin, icariside II, icariin, epimedim C, *osteocalcin*



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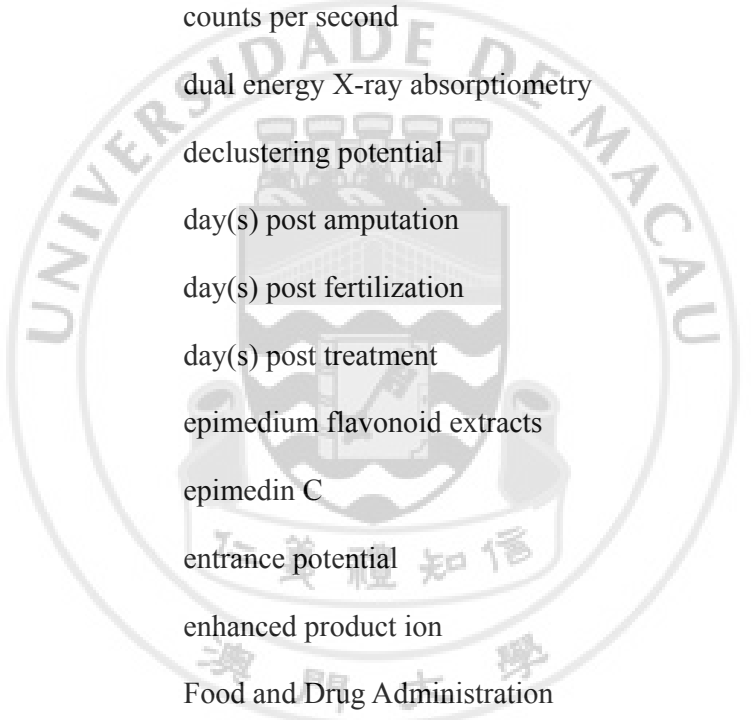
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LIST OF ABBREVIATIONS



AR	alizarin Red-S
ALP	alkaline phosphatase
BSA	Bovine serum albumin
BMP	bone morphogenetic protein
CaSR	calcium-sensing receptor
cps	counts per second
DEXA	dual energy X-ray absorptiometry
DP	declustering potential
dpa	day(s) post amputation
dpf	day(s) post fertilization
dpt	day(s) post treatment
EFE	epimedium flavonoid extracts
ENC	epimedin C
EP	entrance potential
EPI	enhanced product ion
FDA	Food and Drug Administration
GFP	green fluorescent protein
GIOP	glucocorticoid-induced osteoporosis
GLP-2	glucagon-like peptide 2
IC	icariin
IDA	information dependent acquisition
IT	icaritin
IS	icaraside II

MAPKs	Mitogen-activated protein kinases
NF- κ B	nuclear factor kappa-light-chain-enhancer of activated B cells
OCN	osteocalcin
OPN	osteopontin
PFA	paraformaldehyde
PGE2	prostaglandin E2
pH	potential of hydrogen
PTH	parathyroid hormone
RANKL	receptor activator of nuclear factor κ B ligand
RFP	red fluorescent protein
RT-PCR	reverse transcription polymerase chain reaction
SERMs	Selective Estrogen Receptor Modulators