



Assessing the gene expression role of the osteoporotic MG-63 Osteoblasts in the bone formation using cowpea and Vitamin D

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INTRODUCTION

Bone markers are very much essential for understanding the pathology of the disease. Bone, a rigid organ of the body always undergoes 2 continuous phases of remodeling throughout entire life. An imbalance in remodeling causes Osteoporosis. Three markers namely Receptor activator of nuclear Kappa B ligand (RANK-L), Osteonectin (SPARC), Osteopontin (OPN) play a major role in monitoring the status of Osteoporosis. **RANK-L**, member of tumor necrosis factor ligand and Type 2 membrane protein is known for its apoptosis regulatory gene activity and a binding partner of osteoprotegerin and ligand receptor of RANK. **SPARC** also referred as specific protein acidic and rich in cysteine is secreted by osteoblasts during bone formation, initiating mineralization, and promoting mineral crystal formation. **OPN** belongs to family of acidic proteins namely Asp and Glu. Its an extracellular protein and organic component of the bone that binds to calcium and helps in bone mineralization.

AIM

The present aim of the study, is to monitor the gene expression of three markers namely RANK-L, SPARC and OPN using Cowpea extract and Vitamin D by western blot studies.

MATERIALS & METHODS

MG-63 Human Osteosarcoma cell lines were purchased from NCCS pune. EC₅₀ concentrations of Daidzein & genestein (Positive Controls) Whole Cowpea extract and Vitamin D individually and in combinations were exposed to MG-63 Cells for 48 Hrs as per the standardized cell culture techniques.

Western Blot: Cell lysates were taken and blot studies were carried out as per the standardized protocol. Respective RANK-L, SPARC and OPN primary and secondary antibodies were used and blot developed was used finally for determining the expression levels of the markers with respect to control.

RESULTS & DISCUSSION

◆Levels of RANK-L, SPARC and OPN significantly increased to 10 fold with respect to control via western blot. All markers which directly or indirectly having an anabolic effect on the bone metabolism showed significant increase in the expression levels after treatment when compared to control. Thus Cowpea extract and Vitamin D the main experiment source of study provided an anabolic insight in treating the Osteoporosis in the form of natural food diet.

CONCLUSION

Gene expression study through western blot analysis showed the stimulating effects of Cowpea isoflavones along with the vitamin D in the bone formation. Thus supplementation of the whole extract of naturally rich CPIF in the present study provides notch to commercialize Cowpea in the form of capsules and tablets for the treatment of osteoporosis.

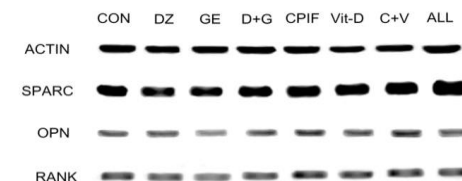
ACKNOWLEDGEMENTS

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TABLES

EXPOSURES	RANK-L	SPARC	OPN
CON	0.76	1.03	0.47
DZ	0.78	0.90	0.57
GE	0.82	0.96	0.59
D+G	0.81	1.07	0.68
CP	0.93	1.24	0.78
V-D	0.98	1.11	0.61
C+V	1.14	1.14	0.84
ALL	1.03	1.40	0.68

GRAPHS



Blots showing the expression levels of protein markers RANK-L, SPARC and OPN relative to structural protein β -Actin for different exposures with respect to control.