Evaluation of the Efficacy and Side Effects of Silymarin Alone and in Combination with Alendronate in the Treatment of Postmenopausal Osteoporosis:

A Pilot Study

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List of Abbreviations

Apaf-1	Apoptotic peptidase activating factor 1.
APR	Acute phase reactivation.
ATP	Adenosine triphosphate.
BAONJ	Bisphosphonates associated osteonecrosis of the jaw.
Bcl-2/Bax	Apoptosis regulator BAX, also known as bcl-2-like protein 4
BMC	Bone mineral content.
BMD	Bone mineral density.
BMP	Bone morphogenetic protein.
BMU	Bone multicellular units.
BPs	Bisphosphonates.
CT	Computerized tomography
DXA	Dual energy x- ray absorptiometry.
E_2	Estradiol.
EE	Ethinyl estradiol.
ER	Estrogen receptor.
ERa	Estrogen receptor α.
FPPS	Farnesyl pyrophosphate synthase.
FSH	Follicle stimulating hormone.
GSH	reduced glutathione
GSSG	oxidized glutathione
HAP	Hydroxyapatite
hBMSCs	Human bone marrow stromal cells.
HDL	High density lipoproteins
IFNɣ	Interferon gamma
IGF1	Insulin growth factor 1.
IL	Interleukin.
LH	Leutinizing hormone.
LS	Lumbar spine.
M-CSF	Macrophage colony-stimulating factor.
MLC	Myosin light chain
MRI	Magnetic resonance imaging
MTE	Milk thistle extract.
OATP	Organic anion uptake transporter peptides
OPG	Osteoprotegerin.
OVX	Ovariectomized.
PARP	poly ADP ribose polymerase enzyme
PPI	Pyrophosphate.
PTH	Parathyroid hormone.
RANK	Receptor activator of nuclear factor-kB.
RANKL	Receptor activator of nuclear factor-kB Ligand.
$RUNX_2$	Runt-related transcription factor.

SBE	Soy bean extract.
SD	Standard deviation.
SERM	Selective estrogen receptor modulator.
SIL	Silymarin.
SIOP	Steroid induced osteoporosis.
TGFB1	Tumor growth factor b1.
TNFα	Tumor necrosis factor alpha
USPSTF	United states preventive services task force.
UVB	Ultra-violet rays B
VEH	Vehicle.
WHO	World health organization.

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Abstract

Background/objective: Osteoporosis is a state in which bone is fully mineralized but its structure is abnormally porous and its strength is less than normal. Post-menopausal osteoporosis is a state in which women at the age of menopause and for the next 10 years lose bone at an accelerated rate, this is mainly due to increased bone resorption, withdrawal of estrogen having removed one of the normal restraints of osteoclastic activity.

Alendronate is classified as a bisphosphonate and currently oral bisphosphonates are the preferred therapeutic option for the treatment of postmenopausal osteoporosis by increasing bone mineral density and decreasing both vertebral and non-vertebral fracture risk.

Silymarin or Mary's thistle is a standardized mixture of four flavonolignans and one isoflavonoid. Silymarin is mainly used as hepato-protectant but in recent studies on ovariectomized rats, it was found that silymarin can be a promising antiosteoporotic pharmacological agent and selective estrogen receptor modulator.

The aim of the study is to evaluate the clinical outcome of combination therapy of alendronate and silymarin on postmenopausal osteoporotic women compared to the monotherapy of each.

Methods: The patients were classified into three groups , the first group received 70 mg alendronate tablet once weekly for two years, the second group received 140 mg silymarin capsule three times daily for two years and the third group received 35 mg alendronate once weekly and 140 mg silymarin three times daily for two years. The bone mineral density of the patients was monitored using dual energy x-ray absorptiommetry (DXA) at the beginning of the study , one year after therapy and 2 years after therapy.

Results: At the end of the study it was found that patients treated with silymarin showed improvement in the mean T-scores of spine and femur and wrist yet this improvement was significantly less than in patients treated with alendronate or combined therapy (p_{spine} =0.000, p_{femur} =0.000 p_{wrist}=0.033).

Conclusion: To reach a conclusion that silymarin is a safe and efficient medication to treat postmenopausal osteoporosis requires further studies with longer time frames, dose assessment and larger group sizes with assessment of patients variables like bone turnover markers, liver functions, kidney functions and thyroid functions.

Introduction

Osteoporosis describes a state in which bone is fully mineralized but its structure is abnormally porous and its strength is less than normal for a person of that age and sex, also there is a significant decrease in bone mass per unit volume of bone tissue and this is accompanied by increased fragility of the bone **(Louis Solomon et al., 2001).**

Postmenopausal osteoporosis affects women at the age of menopause and for the next 10 years lose bone at an accelerated rate (about 3% per year) compared with 0.3% during the preceding decade. This is mainly due to increased bone resorption as the withdrawal of estrogen eliminates one of the normal restraints of osteoclastic activity. In some cases this process is exaggerated and results in osteoporosis and skeletal failure **(Lindsay et al., 1984)**.

There are two types of osteoporosis primary and secondary. Primary osteoporosis is caused by reduction in estrogen in a woman's body after menopause , while secondary osteoporosis is caused by age related changes in the rate of bone building that occurs in both men and women as they grow older. Secondary osteoporosis is also caused by certain medical conditions and treatments as well as by unhealthy behaviors **(Louis Solomon et al., 2001).**

Alendronate is one of the most widely used drugs today. Millions of people have used it and doctors continue to prescribe it even though there are many other osteoporosis drugs on the market. Alendronate can increase bone mineral density and counteract osteoporosis. The doctor might prescribe it for osteoporosis or osteopenia. Alendronate is classified as a bisphosphonate drug like other medications in this class alendronate inhibits bone resorption via action on osteoclasts or on osteoclast precursors **(Lambrinoudaki et al., 2006).** There are many other anti-resorptive agents used in treatment of osteoporosis like selective estrogen receptor modulators (SERMS) and calcitonin.

Silymarin is the most commonly used herbal product by individuals with chronic liver disease since the time of ancient greeks. Silymarin is initially purified out of standard Mary's thistle (silybummarianum) and is a standardized mixture of four flavonolignans (silybinin, isosilybinin, silydianin and silychristin) and the isoflavonoidtoxifolin**(Pepping, 1999).**

Silymarin is an ancient medicinal plant which has been used for centuries for treatment of different diseases such as liver and gallbladder disorders, protecting liver against snake bite and insect stings, mushroom poisoning and alcohol abuse **(Kren et al., 2005).** Recently it has been used in prevention and treatment of cancers **(Chu et al., 2004),** renal protection **(Soto et al., 2004)** and in treatment of Alzheimer disease **(GholamrezaKarimi et al., 2011).** It also has protective effect on the pancreas and immunomodulation effects **(Gharagozloo et al., 2010),** preventing effect against hemolysis **(Psotova et al., 2004)** and protective effect against environmental toxins **(Kiruthiga et al., 2010).** In addition to that silymarin is considered as a promising pharmacological agent as antiosteoporotic and selective estrogen receptor modulator**(GholamrezaKarimi et al., 2011)**so this was taken as a the base of our present study.

This study is aimed to evaluate the clinical outcome of combination therapy of silymarin and alendronate on postmenopausal osteoporotic patients compared to monotherapy of each. This study consists of 69 patients diagnosed with postmenopausal osteoporosis.

Osteoporosis

Osteoporosis is a progressive, systemic chronic skeletal disorder characterized by low bone mass and impaired skeletal micro architecture, resulting in reduced bone strength and increased fracture risk It is a symptomatic until such time as a fracture occurs **(LouisSolomon et al., 2001).**

Bone fractures related to osteoporosis particularly vertebral and hip fractures, cause significant suffering and are associated with high mortality and decreased quality of life **(Cauly, 2000).** The prevalence of fractures related to postmenopausal osteoporosis is very high, indeed osteoporotic fractures are more frequent than the sum of cases of myocardial infarction, breast cancer and stroke. Moreover 20 % of all women experiencing one of these fractures suffer another within a year **(Lindsay et al., 2001)**.

Osteoporotic fractures are three times more common in the vertebrae than in the hip. In addition to fractures, osteoporosis causes deformity, height loss, pain highlyrefractory to treatment, reduced physical activity, respiratorycomplications, limited social activities, dependency upon others, etc. Apart from clinical impairment and worsened quality of life it should be remembered that the financial cost of fully established clinical conditions is substantially greater than the cost associated with the application of effective preventive measures **(Faustina et al., 2003).**

Considering all these factors, the most important goal of osteoporosis therapy is the reduction of fracture risk. There are currently two effective therapeutic strategies for reducing fracture risk:

1. Reducing bone resorption with the use of anti-catabolic agents
2. Increasingbone formation with the use of anabolic agents

Currently potent oral bisphosphonates, such as alendronate, are the preferred therapeutic option for the prevention and treatment of osteoporosis **(Mcclung, 2000)**. They effectively increase bone mineral density and reduce both vertebral and non vertebral fracture risk. Moreover, data from large clinical trials have documented the safety and tolerability of daily bisphosphonates with duration of up to 10 years for alendronate **(Black et al., 2004; Hoskinlg et al., 2004).**

One of the most effective methods for both prevention and treatment of postmenopausal osteoporosis is using oestrogen receptor modulator. It reduces the incidence of vertebral fractures. Based on this fact, this study aims to prove that silymarin can be used in treatment of osteoporosis either alone or in combination with alendronate and this is

because silymarin was proven to act as a selective oestrogen modulator in the femoral metaphysis of ovariectemised rats. **(Bennett et al., 2008).**

Silymarin is initially purified out of standard Mary's thistle (silyburnmarianum) and is a standardized mixture of four flavonolignans (Silybinin, isosilybinin. Sildianin and silychristin) and the iso flavonoid toxifplin**(Pepping, 1999)**.

1-Anatomy and physiology

Bones as structural organs have three main functions: support, protection and leverage. Bone as tissue has an equally important role: it is a mineral reservoir which helps to regulate the composition (and in particular the calcium ion concentration) of the extracellular fluid. For all its solidity, it is in a continuous state of flux, its internal shape and structure changing from moment to moment in concert with the normal variations in mechanical function and mineral exchange. All modulations in bone structure and composition are brought about by cellular activity, which is regulated by hormones and local factors; these agents, in turn, are controlled by alterations in mineral ion concentrations. Disruption of this complex interactive system results in systemic changes in mineral metabolism and generalized skeletal abnormalities **(Solomon et al., 2010).**

Bone Composition

Bone consists of a largely collagenous matrix which is impregnated with mineral salts and populated by cells (osteoblasts and osteoclasts). The matrix Type I collagen fibres make up over 80 per cent of the unmineralized matrix. They form a network which embodies a mucopolysaccharide(proteoglycans) ground substance and also acts as a scaffold on which the mineral component – crystalline hydroxyapatite – is deposited.

Other non-collagenous proteins exist in small amounts in the mineralized matrix mainly sialoproteins (osteopontin), osteonectin, osteocalcin (bone Glaprotein) and alkaline phosphatases. Their functions have not been fully elucidated but they appear to be involved in the regulation of bone cells and matrix mineralization. Osteocalcin is produced only by osteoblasts and its concentration in the blood is, to

some extent, a measure of osteoblastic activity. A number of growth factors have now been identified; they are produced by the osteoblasts and some of them, acting in combination, have a regulatory effect on bone cell development, differentiation and metabolism. Bone morphogenetic protein (BMP) – a collection of growth factor proteins – has attracted a great deal of attention. It was originally found by Marshall Urist in1964 **(Urist, 1965)** and is now produced in purified form from bone matrix. It has been shown to have the important property of inducing the differentiation of progenitor cells into cartilage and thereafter into bone. It is now produced commercially and is being used to enhance osteogenesis in bone fusion operations (**Rihn et al., 2008**).

Bone mineral

Almost half the bone volume is mineral matter, mainly calcium and phosphate in the form of crystalline hydroxyapatite which is laid down in osteoid at the calcification front. In mature bone the proportions of calcium and phosphate are constant and the molecule is firmly bound to collagen. It is important to appreciate that in life demineralization of bone occurs only by resorption of the entire matrix. While the collagenous component lends tensile strength to bone, the crystalline mineral enhances its ability to resist compression. Unmineralized matrix is known as osteoid; in normal life it is seen only as a thin layer on surfaces where active new bone formation is taking place, but the proportion of osteoid to mineralized bone increases significantly in rickets and osteomalacia.

Bone cells

There are three types of bone cell: osteoblasts, osteocytes and osteoclasts.

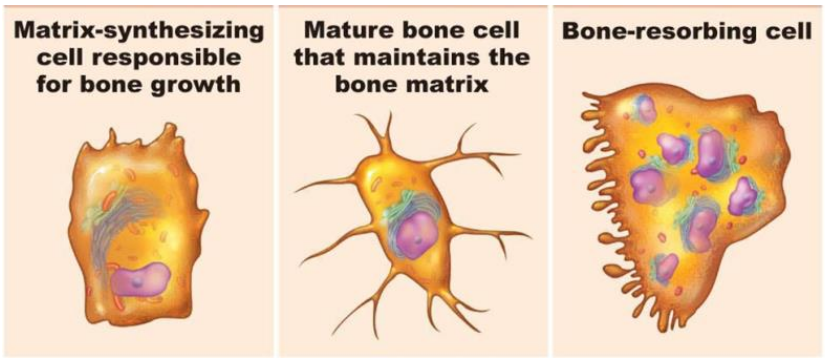

Figure1:a)osteoblasts b)osteocyte and c) osteoclast. (Solomon et al., 2010)