EVALUATION OF EMBELIN FROM EMBELIA RIBES BURM FOR OSTEOPROTECTIVE ASSESMENT

[1] Vitul Kumar, [2] Kokkula Pavan Kumar

[1]M Pharmacy ,[2]Associate Professor,

Faculty of Pharmaceutical Sciences, Mother Hood University, Roorkee-Dehradun Road, Karoundi, Post: Bhagwanpur, Tehsil: Roorkee, Uttarkhand -247661

M Pharmacy,Department of Pharmaceutical Analysis
Aditya Bangalore Institute of Pharmacy education and Research

Corresponding Author:Vitul Kumar
pawan0004@gmail.com

ABSTRACT

A new method was established for evaluation of embelin from Embelia ribes burm for Osteoprotective assessment with effect to three months treatment of Embelia or Vitamin D 3 on Biochemical parameters in serum and urine of OVX rats and also body weight and unterine index of OVX rats with femur physical parameters .With regard to calcium and Phosphorous of S and U all the ALP values are within the range .The length ,weight ,volume and Density of the OVX rats are within the range and reported calulations made with First ,second and third month body weight comparisons of OVX rats by maintain the bone mineral homeostasis .The treatment regimen available produces many side effects due to their influence on hormonal regulation hence this made the herbal field the treatment from plant sources .

KEYWORDS: Embelia ,S Calcium ,S Phosphorus ,Osteoporosis

INTRODUCTION

The systemic degradation of bone, strength, density characterizes osteoporosis, which raise, danger of fragility, risk is an increasing health, economy, which, disease of the bone, which, reservoir of calcium, other metabolites as well as a means of resistance to mechanical forces, mobility, and fracture prevention. The number and quality of the bone affect bone strength. Bone quantity, defined by the structure of the bone as well as by the mineralization, collagen status, overall geometry[1]. Skeletal fracture resistance is greatly influenced by the collagen matrix, mineralization, and the mega and mini-architecture of the bone. Osteoporosis, therefore, are thus characters of low bone strength and microstructural bone degeneration.

With an ageing population, osteoporosis affects about more of white postmenopausal women; this proportion is anticipated to rise significantly in the near future. Osteoporosis patients have a 40 % fractures, although fractures effect the spine, or wrist, impact other bones, ribs[2]. Age, prior fractures, glucocorticoids, are a few warning signs that need to be considered.

Because elderly people may also be suffering from conditions that garner more attention, such as cancer or cardiovascular disorders, for the early identification of osteoporosis. Using diagnostic test kits, the serum was examined, presence of. With the aid of diagnostic assay kits. The right femur's right bone density and volume were additionally determined using Archimedes' methods. The currently underway investigation also detected the histology of left femur.

MATERIALS AND METHODS:

MATERIALS:

so	**Reagent**	
1	Vitamin D_3	H.media, Mumbai
2	Calcium estimation kit	Erba diagnostic kits
3	Phosphorous estimation kit	Erba diagnostic kits
4	Alkaline phosphatase kit	Erba diagnostic kits
5	Conc.Hcl	S.D.Finechemicals, Mumbai
6	Formaldehyde	Associate chemicals, Mumbai
7	Tween 80	Himedia, Mumbai

Chemicals and Reagents:

Sl. No	**Name of the Apparatus**	**Supplied By**
1	Auto analyzer (Qualisystems AR-601)	GSK- Mumbai
2	Electronic Digital Balance	Essae teroka Ltd, Mumbai
3	Cold centrifuge	RM Ltd, Mumbai
4	D. frost	BS Industries, Bangalore
7	Microplate reader	Biotek

METHOD DEVELOPMENT

Isolation of Embelin

Finely grounded berries of *embelia ribes* (2 kg), exhaustively, with *n*-hexane by cold

extraction method. After 48 h, the solvent was decanted, distilled off boiling water. The extract so obtained was concentrated *in vacuo* and subjected to column chromatography (120 - 200 mesh). The purity o\was checked by comparison, sample of embelin over TLC plate as well as by mixed melting point method.

Embelin

Using Tween 80, embelin, suspended in distilled water.

Serum and Urine analysis:

The biochemical analysis of Ca, P & alk phosphatase in serum samples and analysis of calcium, phosphorus in urine samples were done using micro plate reader with available reagent kits.

Calcium estimation:

Calcium present in samples of serum, urine and bone ash were estimated by using Erba reagent kit

O-Cresolphthalein Complexone + Ca^{++} Alkaline solution → Purple colored complex.

Assay: Table no 1 Results of serum and urine analysis

Pipette	**B**	**S**	**T**
Working reagent.	1000µl	1000µl	1000µl
Dist. H_2O	10µl	_	_
S.	_	10 µl	_
T.	_	_	1 µl

i. Phosphorus estimation:

Phosphorus present in samples of serum, urine and bone ash were estimated by using Erba reagent kit (Ammonium Molybdate Procedure).

Strong acids

Phos.p + amm. molybdate → phosphomolybdate

Table no 2 Results of Phosphorus estimation

Pipette	**B**	**S**	**T**
Reagent.	1000µl	1000µl	1000µl
Dist. H_2O	20 µl	_	_
S.	_	25µl	_
T.	_	_	25µl

RESULTS [13]

Effect of three months treatment of Embelin or $VitD_3$ on biochemical parameters in serum and urine of OVX rats

Effect of Embelin, Ovariectomy to rat significantly increased the S-Ca, S-PO_4 ($p<0.0001$, P 0.0001,) and SALP (P 0.001), simultaneous with Embelin significantly reverse the increased level of S-Ca, S-PO_4 (P 0.005, P 0.001) S-ALP(P 0.01) activity induced by ovariectomy treatment with standard drug $VitD_3$ also significantly reduced the ovariectomized induced increase of S-Ca, S- PO_4 ($P<0.001$, $P<0.0001$) and S-ALP (P 0.0001)activity. In addition ovariectomy significantly increased U-Ca, U-PO_4 ($P<0.001$, $P<0.0001$) excretion when compared to Sham operated group. Embelin was comparable with the standard $VitD_3$.

Effect of three months treatment of Embelin or $VitD_3$, body mass & uterine effect of OVX rat

Daily food consumption in each group was the same. In the current study, all 6 groups' initial body weights were similar, and the body weight of ovariectomized rats increased continuously despite this. Embelin significantly reduced the ovariectomized-induced increase.

As expected, removal of ovary (P 0.001) reduced uterus weight in ovariectomised, Oral embeline in dose, 10 and 20mg/kg body weight ovariectomised g (P 0.01). As compare to sham operated group ($P<0.001$) treatment embeline significantly (P 0.01) improved loss uterine weight induced by ovariectomy when compare to ovariectomised rats. Chronic treatment with $VitD_3$ for ovariectomised rats significantly ($P<0.001$) improve the loss of uterine index.

Effect of three months treatment of Embelin or $VitD_3$ on femur physical parameters of OVX rats

The ovariectomized rats, (P 00.01, P 00.001) reduction in femur physical parameter such as femur weight, length(mm), volume(ml), density(g/cm^3) it is due to loss of mineral level in the bone and estrogen deficiency. The oral administration of embelin has shown the bone length, weight, volume, density when compare to ovariectomized rats. Simultaneously standard $vitD_3$ administration to ovariectomized rats reduced weight, length, density, volume of bone when compare to ovariectomized rats.

Effect of three months treatment of Embelin or $VitD_3$ on Ash parameters of OVX rats

The effect of embelin or $vitD_3$ on ash parameters of ovariectomized rats has shown in table no.3 the results showing that the ovariectomized rats has shown extremely significant reduction ash weight ($P <0.001$). But the treatment with embelin for ovx rats, which shown

ash weight (P<0.05), to ovariectomized rats and also treatment with standard vitD_3 which restore the decreased ash weight.

When comes ash calcium and phosphorus content (P<b0.001, P<b0.01) dose of 10 & 20mg /kg body mass. As well as treatment with standard vitD_3 which shown significantly (P<0.001) increase in ash ca and p content.

Table: 3 Embelin or vitamin D3 administration for 3 months and its impacton the biochemical markers in the serum and urine of OVX rats

Treatment groups (mg/kg, p.o.)	S-Calcium (mg/dl)	S-Phosphorus (mg/dl)	U-Calcium (mg/dl)	U-Phosphorus (mg/dl)	ALP (IU/L)
Sham Group	1.649±0.119***	2.546±0.0879***	1.923±0.0574**	173.2±10.73***	1053±67.23**
OVX Control	2.765±0.112	3.336±0.1385	3.455±0.2174	249.5±6.530	1643±147.7
OVX +E-10	2.079±0.1524*	2.841±0.09094*	2.390±0.2715*	178.4±3.955***	1023±29.04**
OVX+E-20	1.795±0.2599**	2.839±0.1097*	2.408±0.1989*	165.7±5.870***	1119±111.8**
OVX+Vit D3	1.828±0.1539**	2.281±0.06005***	1.835±0.2865***	170.4±8.534***	708.1±8.362***

Table: 4 Effect of Embelin or Vitamin D3 administration for 3 months

Treatment groups (mg/kg, p.o.)	First month	Second month	Third month	Uterine index (mg/g BW)
Sham Group	193.3±3.333	207.5±2.500***	215.8±2.007***	1.564±0.1174***
OVX Control	208.3±4.773	238.3±4.773	273.3±4.944	0.2925±0.0207

OVX +E-10	198.3±4.773	208.3±4.773***	220.8±4.167***	0.5769±0.0200**
OVX+E-20	200.0±5.774	210.0±5.774**	212.5±5.737***	0.5753±0.0311**
OVX+Vit D3	196.0±5.099	200.0±3.162***	204.0±1.871***	0.7353±0.0631***

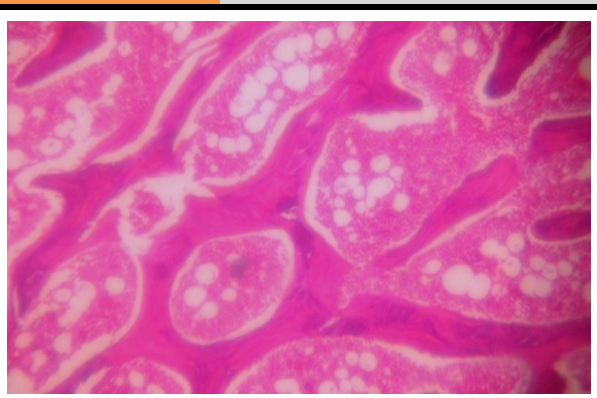
Table:4 Effect of Embelin or Vitamin D3 treatment for 3 months on the physical characteristics of the OVX rats' femurs.

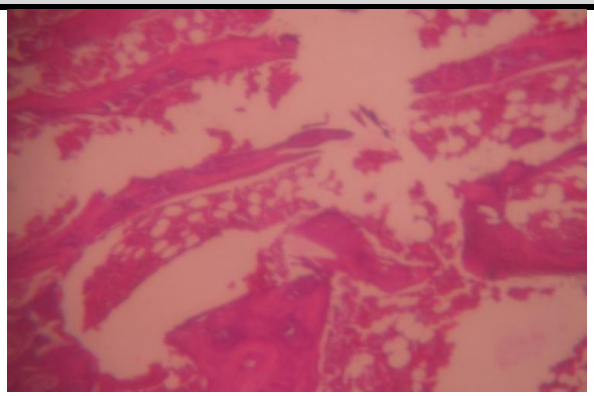
Treatment groups (mg/kg, p.o.)	Length (mm)	Weight(g)	Volume(ml)	Density(g/cm$^{3)}$
Sham Group	32.63±0.126**	0.5367±0.01856***	0.7350±0.02363***	0.8383±0.04557***
OVX Control	30.29±0.097	0.2733±0.01801	0.4660±0.01077	0.4467±0.0105
OVX +E-10	33.01±0.574***	0.4442±0.02740***	0.6900±0.03674**	0.7767±0.03533***
OVX+E-20	32.27±0.326*	0.4130±0.01538**	0.6000±0.06124	0.7600±0.05083***
OVX+Vit D3	33.67±0.456***	0.4580±0.03430***	0.7040±0.02040**	0.7392±0.04522***

Table: 5 Effect of Embelin or vitamin D3 treatment for 3 months on the OVX rats' ash parameters

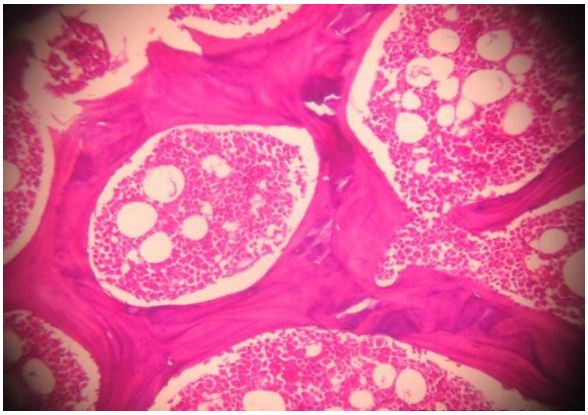
Treatment Groups (mg/kg, p.o.)	A weight(g)	% A	A Ca(mg/dl)	Ash Phosphorous (mg/dl)
Sham Group	0.339±0.020***	59.93±1.92***	4.032±0.75***	1.925±0.03**
OVX Control	0.229±0.006	96.42±4.99	0.825±0.04	0.837±0.05

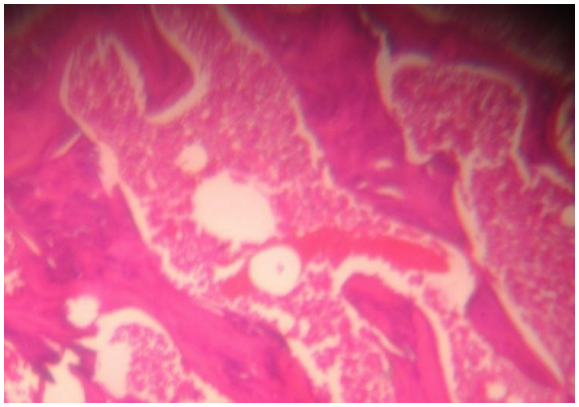
OVX +E-10	**0.289±0.005***	**62.90±3.25*****	**3.086±0.21****	**2.165±0.21*****
OVX+E-20	**0.294±0.016***	**71.13±2.73****	**2.088±0.17**	**1.860±0.14****
OVX+Vit D3	**0.324±0.018*****	**67.45±6.66****	**2.718±0.11****	**2.201±0.24*****





Normal **Ovx**





Embelin 10 Embelin 20

Fig no 1 : Comparison of Normal,Ovx ,Embelin 10 and 20 with Vitamin D 3

DISCUSSIONS[14]

The development of an analytical method for the determination of drugs by HPLC has received considerable attention in recent years because of their importance in quality control of drugs and drug products. The objective of this study was to develop a simple, rapid, precise, accurate and sensitive HPLC method for the analysis of sumatriptan in bulk and its pharmaceutical dosage form by using solvent system of TEA : ACN : methanol in the ratio 80:10:10 and C_8 ODS Inertsil (250*4.6mm, 5µi.d) stationary phase. The chromatographic condition is set at flow rate of 1ml/min with PDA detector at 221 nm. As per ICH requirements validation studies are carried out by using freshly

prepared solutions. The main components of bone tissue, a complex and metabolically active organ, are calcium and phosphate salts. They serve as a pool for extracellular calcium and are crucial for maintaining skeletal mechanical integrity and appropriate skeletal growth[162]. Any modification in the bone minerals due to menopause, glucocorticoid administration, hormonal imbalance and other disease conditions may lead to skeletal disorders.

Accompanying structural alterations make the bone more brittle and predispose it to fracture. The current strategy for creating anti-osteoporotic medications focuses on the two primary mechanisms.

As a result, oestrogen insufficiency in humans increases plasma calcium levels. The side effects of the most commonly prescribed anti-osteoporotic medications include thromboembolism (HRT), esophageal irritation, abdominal or musculoskeletal pain, nausea, and heartburn (Bisphosphonates), as well as anorexia, (calcitonin). Alternative methods of controlling osteoporosis are therefore required. A growing number, people are interested in using phytoestrogens to treat and prevent osteoporosis. The preferred method for treating postmenopausal osteoporosis is anabolic treatment.

Embelin has also been demonstrated to prevent osteoclastogenesis in vitro caused by tumour cells and cell signalling pathway.[20] Embelin may be involved in, according to such in vitro findings, purpose of the current investigation is to ascertain how Embelin affects rats with experimentally induced osteoporosis.

The calcium, phosphorus, ALP was evaluated were analyzed using microplate reader in ovariectomized rat. The embelin has shown decreased calcium level in urine, increase in serum and increased ash calcium level and has no effect on phosphorus levels and decreased ALP levels in serum and urine respectively. The embelin of 10 mg/kg has shown significant effect similar to standard vitamin D_3 in all parameters.

The calcium levels decreased in urine The ALP levels decreased in serum may be due to osteoblastic activity through new bone formation. Bone density was increased by embelin of 10 mg/kg dose.

In histopathological examination of ovariectomized induced osteoporosis in rats, the embelin of dose 10 mg exhibited when observed for ossification. There was change in structural pattern of profound increase in connectedness, intact bone lamellae and thickness of trabecular bone formation. This has shown significant effect with 10 mg of embelin compared to 20 mg of embelin. This may due to increased calcified cartilaginous deposits.

CONCLUSION

The embelin isolated from *Embelia ribes* Burm.berries possess the osteogenic property by stimulating the new bone mass formed and mineralize contents. In current assay embelin, found effective in stimulating the new bone formation. Activity of embelin may be due to the presence of phytoestrogens which may act by binding to the estrogen receptors and they're by maintaining the bone mineral homeostasis. Further study on preclinical in animals and clinical studies, the use of human volunteers could result in the creation of a novel osteoporosis treatment.

CONSENT AND ETHICAL APPROVAL

It is not applicable

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COMPETING INTEREST

Authors have declared that no completing interests exists

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