Bony adverse effects are amongst the potentially adverse clinical consequences with antiepileptic drugs (AEDs). Despite this, there is limited data on the effect of anti-osteoporotic therapies on AEDs-induced bone loss. We hypothesize that estrogen deprivation following AED therapy could lead to adverse bony effects. AEDs inhibit human aromatase enzyme and stimulate microsomal catabolism of estrogens. Estrogen deficiency states are known to reduce the deposition of transforming growth factor-β (TGF-β3), a bone matrix protein, having anti-osteoclastic property. Thus, an attempt was made to investigate the effect of raloxifene (RLX), a selective estrogen receptor modulator, in comparison with calcium and vitamin D3 (CVD) supplementation, on AEDs-induced alterations in bone in mice and to unravel the role of estradiol and TGF-β3 in mediation of bony effects by either AEDs or raloxifene. Further, the effect of raloxifene on seizures and on the antiepileptic efficacy of these AEDs was also investigated.

Thus, the present study was designed with the following aims and objectives:

- To evaluate the comparative effect of three AEDs (phenytoin, sodium valproate and levetiracetam) on bony alterations in female mice
- To investigate the effect of raloxifene (in comparison with calcium and vitamin D3 (CVD) supplementation) on AEDs-induced alterations in bone mineral density and bone turnover markers
- To study the effect of raloxifene on the antiepileptic efficacy of AEDs in electroshock-induced seizures
- To assess the probable role of estrogen and TGF-β3 in mediation of bony effects by either AEDs or raloxifene

Swiss strains of female mice were treated with PHT (35 mg/kg, p.o.), SVP (100 mg/kg & 300 mg/kg, p.o.) and LTM (100 mg/kg & 200 mg/kg p.o.) for 4 months to determine effects on bone. The plasma concentrations of AEDs were determined to correspond with clinically relevant therapeutic ranges. The doses of RLX and calcium and vitamin D supplements (CVD/CVDD) were derived from converting the doses used clinically in humans. Preventive treatment (RLX or CVD) was administered concurrently with AEDs for 4 months while therapeutic treatment (RLX or CVDD) was started for 1 month after 4 months treatment with AEDs. Bony changes were assessed by histopathology of femur and lumbar vertebral bones.
and by determination of bone mineral density (BMD) and bone mineral content (BMC) in these bones using Dual energy X-ray absorptiometry (DEXA). Further, bone turnover markers including alkaline phosphatase (ALP), tartarate resistant acid phosphatase (TRAP) and hydroxyproline (HxP) were determined in lumbar bones and urinary excretion of calcium was measured. Serum estradiol levels and lumbar TGFβ3 levels were also determined.

The findings of the present study may be summarised as below:

I. Comparative effects of three AEDs on bony alterations/ development of AEDs-induced bone loss model in female mice

Treatment with PHT (35mg/kg) and SVP (300 mg/kg) for 4 months induced bone loss in mice as evidenced by reduced bone mineral density in femur and lumbar vertebrae. The findings were supported by alterations in BTMs in lumbar bones including reduced alkaline phosphatase activity (markers of bone formation), increased TRAP activity and reduced HxP content and increased U-Ca excretion. Histopathological findings of femoral and lumbar bone also provided evidence of bone loss by presence of osteoclasts, ruffled border and rarefaction of bone matrix. These changes were observed at doses that attained the plasma drug concentration well within the recommended human therapeutic ranges.

*Phenytoin and sodium valproate could be used as a model of bone demineralization in Swiss albino female mice*

Treatment with LTM (100 mg/kg & 200 mg/kg p.o.) for 4 months failed to produce any change in either histopathology, BMD, BMC in femur and lumbar vertebrae or bone turnover markers in lumbar bones.

*LTM may be a safer alternative as compared to PHT and SVP in terms of AEDs-induced adverse effects on bone and can be preferred in epileptic females who are prone to osteoporosis or having a risk factor for osteoporosis*

II. Preventive and therapeutic treatment with raloxifene on PHT and SVP-induced bone loss and comparison with CVD/CVDD

Both preventive and therapeutic treatment with RLX (15 mg/kg p.o.) significantly reverted PHT and SVP-induced reduction in BMD and BMC. Though CVD/CVDD also reversed
PHT and SVP-induced reduction in BMD, the effect was less pronounced as compared to RLX.

*RLX can prevent or treat PHT and SVP-induced bone loss and is more efficacious than CVD/CVDD in preventing or treating AEDs-induced bone loss*

Treatment with PHT (35 mg/kg p.o.) and SVP (300 mg/kg p.o.) significantly (p<0.001) reduced the bone alkaline phosphatase (ALP) activity in the lumbar vertebrae. While RLX, CVD, CVDD per se did not show any significant change in lumbar ALP activity as compared to control group, they significantly restored the AEDs-induced reduction in bone ALP levels, RLX being more effective as compared to CVD/ CVDD.

*Increased lumbar ALP activity by RLX point towards its positive role in enhancing bone formation in PHT and SVP-induced bone loss*

TRAP activity was significantly elevated (p<0.001) in the lumbar vertebral bones of mice treated with PHT (35 mg/kg) and SVP (300 mg/kg) in both preventive and therapeutic groups. RLX and CVD/CVDD effectively mitigated the elevated TRAP levels in both preventive and therapeutic groups without significantly affecting the lumbar TRAP activity when administered alone.

*RLX can prevent or treat TRAP-catalyzed osteoclastic bone resorption*

The hydroxyproline (HxP) content in lumbar vertebra was significantly reduced in PHT (35 mg/kg) and SVP (300 mg/kg) treated groups as compared to control (p<0.001). In the groups receiving either preventive or therapeutic treatment with RLX, CVD and CVDD, HxP levels were elevated, RLX being more effective as compared to either CVD/ CVDD. There was no significant change in the lumbar HxP content when these treatments were administered alone.

*RLX could improve AEDs-induced reduction in bone’s mechanical strength*

PHT (35 mg/kg) and SVP (300 mg/kg) treatment significantly (p<0.001) increased the urinary excretion of calcium (U-Ca) as compared to control. Preventive and therapeutic treatment with RLX significantly reduced the urinary Ca$^{2+}$ excretion (p<0.001). Similar effects were obtained with CVD/ CVDD though the effects were milder (p<0.05) as compared to RLX.
Summary and Conclusion

**III. Effect of raloxifene on seizures and on antiepileptic efficacy of AEDs**

PHT at 35 mg/kg, SVP at 300 mg/kg afforded 100% and LTM at 200 mg/kg afforded 75% protection against electroshock seizures as evidenced by complete abolition of the tonic hind limb extension (HLE). Combination groups of AEDs with RLX were unable to produce any significant change in the tonic HLE observed during seizure activity and elicited responses similar to effects observed with *per se* treatment of AEDs. Raloxifene did not affect the antiepileptic activity of either of the AEDs. Though it increased the latency to tonic HLE and reduced the duration, the effects were not statistically significant.

*RLX does not alter the antiepileptic efficacy of AEDs ruling out any possible pharmacodynamic interaction of RLX with these AEDs on chronic use.*

On the basis of II and III above, it can be deduced that:

*Raloxifene could be considered along with PHT and SVP to prevent or cure bone loss*

**IV. Effect of chronic treatment with AEDs on serum estradiol levels and the effect of RLX on the same**

Chronic treatment with PHT (35 mg/kg) and SVP (300 mg/kg) significantly reduced serum estradiol levels in mice (p<0.001). Preventive (RLX and CVD) and therapeutic (RLX and CVDD) treatment could restore the reduced serum estradiol levels though RLX was better than CVD in the preventive group. No significant change in the serum estradiol was observed when either of the treatments (RLX, CVD/CVDD) was administered alone.

*Reduced estradiol could possibly contributed to PHT and SVP induced bone loss*

Chronic LTM (200 mg/kg p.o) administration for 4 months did not affect serum estradiol levels.

*LTM did not affect estradiol levels and did not produce bony altertations*

**V. Effect of chronic treatment with AEDs on lumbar transforming growth factor β3 (TGF β3) and the effect of RLX on the same**
Chronic treatment with PHT (35 mg/kg) and SVP (300 mg/kg) significantly reduced TGF-β3 content of lumbar bones of mice (p<0.001).

**PHT and SVP induced depletion of estradiol was associated with downregulation of TGF β3 level in lumbar bones which possibly contributed to bone loss**

The lumbar TGF-β3 content (but not estradiol) was significantly reduced after chronic LTM (200 mg/kg p.o) administration for 4 months as compared to control group.

**Reduced TGF-β3 doesn’t appear to be a consequence of depleted estradiol in case of LTM and might not have played a major role in mediating effects on bone when not accompanied with estradiol depletion**

Preventive and therapeutic treatment with RLX normalized the reduced lumbar TGF-β3 content induced by PHT and SVP. CVD and CVDD also mildly reverted PHT and SVP-induced reduction in lumbar TGF-β3 though a statistically significant change was observed only in SVP (and not PHT) treated group. No significant change was observed when either of the treatments (RLX, CVD/CVDD) was given alone.

**The bone protective effects of Raloxifene could be mediated partly, by direct estrogenic action and increased estrogen level and partly, by upregulating TGF-β3 expression in bone**

To conclude, our study extends previous reports of adverse effects of AEDs on bone and report the same in Swiss strain albino female mice for the first time. Further, the lack of bony effects observed following LTM treatment suggests that the same could be a better alternative to PHT or SVP in female epileptic patients or those having a risk factor for osteoporosis. Further, deprived estrogen levels (that in turn reduced lumbar TGF-β3 content) following PHT and SVP, might represent one of the various mechanisms of AEDs-induced bone loss. Raloxifene preserved the bony changes without interfering with antiepileptic efficacy of these drugs, and hence raloxifene could be considered a potential therapeutic option in the management of PHT and SVP-induced bone disease if clinically approved.